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Anticancer Activity of Methanol Extract of Green Tea against Cervical Cancer

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ABSTRACT

Identification and development of natural products used for cancer prevention have attracted a lot of attention globally. Herbal extracts with their proven potential and less side effects in therapeutics have replaced the synthetically derived drugs in modern allopathic medication system. In a low resource country like India, an inexpensive dietary chemo-preventive intervention would be an attractive adjunct to existing cervical cancer prevention programs. It is observed that the studies on the relationship between cervical cancer and green tea are limited, and the observational data are very rare. Hence, in this study an attempt was made to find out the efficacy of methanol extract of green tea against cervical cancer cells (HeLa) by in vitro model systems; and to study the DNA repair capacity of green tea extracts on cultured lymphocytes of cervical cancer patients. It was found that the green tea decreased cell viability in malignant cells in a concentration dependent manner and the IC50 value of green tea extract was found to be 111.9 µg/ml. Chromosomal aberration studies indicate that methanolic extract of green tea showed a reduction in the number of Chromatid Type Aberration (CTA) in the experimental samples when compared to control samples. Hence, it may be concluded that since green tea could cause cell death in HeLa cells and reduce the DNA damage in cultured lymphocytes of cervical cancer patients it can be considered as a potent anticancer agent against cervical carcinogenesis.

Keywords: Green tea, DNA repair, HeLa, Cervical cancer, Chromatid Type Aberration.

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Introduction

In the developing world, cervical cancer remains a common malignancy impacting the lives of women during their highest productivity period. Cervical cancer has continuously been striking hard on the poorest countries such as Central and South America, the Caribbean, Sub-Saharan Africa, some parts of oceans and Asia with rates as high as 30 per 100,000 women, compared to North America and Europe that have reports of about 10 per 100,000 cases. There are approximately 1.4 million women worldwide living with cervical cancer and India may account for more than one-fourth of the total, reporting nearly 132,000 new cases annually. In a low resource country like India, an inexpensive dietary chemopreventive intervention would be an attractive adjunct to existing cervical cancer prevention programs. Numerous environment and host factors, some of which are known and many unknown, contribute to cancer development. Repeated exposures of small doses of natural toxicants may lead to hazardous situations (Iverson, 1991). Oxidative damage to DNA promotes mutation; thus enhancing the risk of carcinogenesis. It is difficult to eliminate mutagenic/carcinogenic factors present in our environment; however it is possible to eliminate the risk of cancer through simple dietary and herbal recommendations (Block *et al.*, 1992).

Herbal extracts with their proven potential and less side effects in therapeutics have replaced the synthetically derived drugs in modern allopathic medication system (Sakthivel and Guruvayoorappan, 2012). Evidence has been provided that dietary phytochemicals may play important roles as chemopreventive or chemotherapeutic agents in the prevention of many diseases; possesses antimutagenic effects; and modulate and stimulate the immune system (Raskin *et al.*, 2002; Rates, 2001) which in turn results in normal functioning of the whole body.

Green tea has been considered as a healthful beverage since ancient times. The plant of tea (*Camellia sinensis*) has been grown in South East Asia for thousands of years and is now cultivated in more than 30 countries around the world. Its consumption has reached a point where it has become the second most commonly consumed beverage worldwide. This popularity was due to its characteristic aroma, flavor and most influencing health benefits (Ahmad *et al.*, 1998; Harbowy and Balentine, 1997). The term 'green tea' refers to the product manufactured from fresh tea leaves by steaming or drying at elevated temperature with the precaution to avoid oxidation of polyphenolic components (Chow and Kramer, 1990). Like most herbs, the precise composition of green tea varies with the geographic origin of the leaf, the time of harvest and processing techniques.

Green tea has been shown to inhibit the occurrence of many different types of cancers (Zou *et al.*, 2002; Mao *et al.*, 2011; Khan *et al.*, 2012; Nguyen *et al.*, 2012). In many animal studies, the polyphenolic fraction isolated from green tea, the water extract of green tea, or individual polyphenolic antioxidants present in green tea have been shown to provide protection against chemically induced carcinogenesis in lung, liver, oesophagus, forestomach, duodenum, pancreas, colon and breast (Ahmad *et al.*, 1998; Katiyar and Mukhtar, 1996). Green tea contains several antioxidant compounds, known as polyphenols which plays a key role in diseases

Epigallocatechin gallate (EGCG), epicatechin gallate (ECG), epigallo catechin (EGC) and epicatechin (EC) are some of the polyphenol compounds found in green tea. These polyphenols account for the pungency and unique flavor of green tea. Catechins and other polyphenols present in green tea are antioxidant and anti-inflammatory in nature and have been shown to possess anti-carcinogenic activity (Baliga and Katiyar, 2006).

The biological activity of green tea is due to different catechins. EGCG has been identified as the principal antioxidant contributing to approximately 30 per cent of the total antioxidant capacity of green tea and has been recognized as the major and potentially effective chemopreventive agent present in green tea leaves (Stewart *et al.*, 2005; Ahmad *et al.*, 1998; Katiyar and Mukhtar, 1996). It has been assumed that EGCG induce apoptosis and promote cell growth arrest by altering the expression of cell cycle regulatory proteins and Bax/BCl_2 function, activating killer caspases, and suppressing nuclear factor kappa-B function. EGCG modulates the signal transduction pathways involved in cell proliferation, transformation, inflammation, apoptosis and metastasis (Khan *et al.*, 2006; Na and Surh, 2006 and Gupta *et al.*, 2004). EGCG induces growth arrest and apoptosis through multiple mechanisms and can be used for cancer prevention, mainly pancreatic cancer (Shankar *et al.*, 2007 b). EGCG could induce apoptosis *in vivo* in sarcoma 180 cells through alteration in G 2/M phase of the cell cycle by up-regulation of P 53, bax and down regulation of C-myc, bcl-2 and UIB, U4-U6 USNRNAs (Manna *et al.*, 2006). According to Ran *et al.* (2007) EGG inhibits DNA replication and consequently induces leukemia cell apoptosis of the human gastric cancer cell line MKN 45 and the effect is in a time and dose dependent manner.

The apoptotic pathway triggered by EGCG in MKN 45 is mitochondrial dependent. EGCG might prevent alveolar bone desorption by inhibiting osteoclast survival through caspase-mediated apoptosis (Yun *et al.*, 2007). EGCG treatment has a dose dependent effect on ROS generation and intracellular ATP levels in MCF-7 cells, leading to either apoptosis or necrosis; with the apoptotic cascade involving JNK activation, Bax expression, mitochondrial membrane potential changes and activation of caspase-9 and caspase-3 (Hsuw and Chan, 2007). Green tea has been shown to inhibit the occurrence of cancers (Zou *et al.*, 2002; Mao *et al.*, 2011; Khan *et al.*, 2012; Nguyen *et al.*, 2012) such as gastric, breast and prostate. The protective effects of EGCG are due to its ability to decrease lipid peroxidation, oxidative stress, and the production of nitric oxide (NO) radicals by inhibiting the expression of inorganic nitric oxides. It also ameliorates the over production of pro-inflammatory cytokines and mediators, reduces the activity of NF-kappa-B and AP-1, and the subsequent formation of peroxynitrite with NO and reactive oxygen species (Tipoe *et al.*, 2007). It also protects cellular damage by inhibiting DNA damage and oxidation of LDL and has many putative health benefits. Numerous mechanisms have been proposed to account for the cancer preventive effects of green tea and EGCG in laboratory animal models. These mechanisms include the inhibition of - growth factor signaling, key cellular enzymes, gene transcription and the induction of tumor suppressor genes (Yang *et al.*, 2006; Khan *et al.*, 2006; Chen *et al.*, 2004; Tachibana, 2009).

During the last decade, the relationship between tea consumption and cancer has been of research interest for many investigators. Recent reports have thoroughly reviewed and summarized epidemiological and experimental studies on tea and cancer prevention (Johnson, 2007; Lee *et al.*, 1995; Mukhtar and Ahmad, 1999; Yang and Wang, 1993; Yang *et al.*, 1998), especially tea compound in inhibition of cervical carcinogenesis (Ann *et al.*, 2003; Ahn *et al.*, 2003; Issa *et al.*, 2007; Li *et al.*, 2005; Yokoyama *et al.*, 2004). Rosengren (2003) indicated that the green tea catechins reduced the proliferation of breast cancer cells *in vitro* and decreased breast tumor growth in rodents. Mittal *et al.* (2004) reported that the treatment with EGCG decreased cell viability at different stages studied in human breast carcinoma MCF-7 cells, but had no adverse effect on the growth of normal mammary cells. Zang *et al.* (2002) reported that ovarian cancer risk declined with increasing frequency and duration of green tea consumption. Yu *et al.* (2004) reported that EGCG inhibited the growth of prostate cancer adenoma cells and induced apoptosis. However, studies of the relationship between cervical cancer and green tea are limited, and the observational data are very rare. In this study, an attempt was made to find out the cytotoxic effect of green tea on cervical cancer cells (HeLa).

In the last few decades, studies have shown that cell lines can serve as an initial screen for agents that might regulate drug resistance. The utility of cell lines acquired from tumor allows the investigation of tumor cells in a simplified and controlled environment (Arya *et al.*, 2011).

In recent years, after realizing the high antioxidant content of green tea which offer protection against the occurrence of cancer, studies evaluated the effects of green tea extracts on different cell lines. Several mechanisms of action were detected. Specifically in HeLa cells, pre-incubation of the ethanol extract of green tea showed an IC50 of 256μg/ml and its mechanism of action is associated with the inactivation of cytosolic thioredoxin reductase (Wang *et al.*, 2008). Green tea also affects the function of VEGF and hypoxia-inducible 1α (HIF-1α) in HeLa cells and inhibits HeLa cell proliferation upon irradiation with a laser light (Sommer *et al.*, 2010). Green tea extract also decreases the cell proliferation of breast cancer cell line MDA-MB231 (Sartippour *et al.*, 2002). Previous investigations have shown that polyphenols inhibited proliferation and cell death.

The use of chromosomal alterations as markers of early biological effects is well established in genotoxicity studies. A relationship between chromosomal damage and cancer development has been suggested since the beginning of the 20th century, but only since 1960 have extensive data been gathered on the frequency of Chromosomal Alterations (CAs) in Peripheral Blood Lymphocyte Culture (PBLC) of humans exposed to known or suspected genotoxic carcinogens. The idea of causal association between chromosomal alterations and cancer risks was based on the concept that genetic damage in lymphocytes reflects similar damage in cells undergoing carcinogenesis is the basis for this study.

Materials and Methods

Collection and Preparation of the Samples

Fresh tea leaves were collected from Valpparai Tea Estate, Coimbatore District and were steamed rolled, dried, powdered using an electrical grinder and sieved to obtain a fine powder

Soxhlet Extraction

10 g of the powder was weighed and petroleum ether, chloroform, methanol and water were used as solvents for soxhlet extraction in the increasing order of polarity. The distillation process was carried out at a low temperature of 40°C. After evaporation of solvents, corresponding residues were obtained and stored in the refrigerator for further use.

Preparation of Extracts

100mg of the soxhlet extract was dissolved in 2ml of Dimethyl Sulfoxide (DMSO) and then mixed with 100ml distilled water and which formed a 1000ppm solution. From the stock solution, solutions of required concentration were prepared and used in this study.

Antitumor Activity of Green Tea

Cell Proliferation assay (MTT) in HeLa Cells

The human cervical cancer cell line (HeLa) was obtained from National Centre for Cell Science (NCCS), Pune and grown in Eagles Minimum Essential Medium (EMEM) containing 10 per cent Fetal Bovine Serum (FBS). All cells were maintained at 37°C, 5 per cent CO_2, 95 per cent air and 100 per cent relative humidity with weekly passages.

Cell Treatment Procedure

The monolayer cells were detached with trypsin-ethylenediaminetetraacetic acid (EDTA) to make single cell suspensions and viable cells were counted using a hemocytometer and diluted with medium containing 5 per cent FBS to give a final density of $1x10^5$ cells/ml. One hundred microlitres per well of cell suspension were seeded into a 96-well plate at a plating density of 10,000 cells/well and incubated to allow for cell attachment at 37°C, 5 per cent CO_2, 95 per cent air and 100 per cent relative humidity. After 24 hours the cells were treated with serial concentrations of the MEGT. They were initially dissolved in dimethyl sulfoxide (DMSO) and diluted to twice the desired final maximum test concentration with serum free medium. Additional four, 2 fold serial dilutions were made to provide a total of five sample concentrations. Aliquots of 100µl of these different sample dilutions were added to the appropriate wells already containing 100µl of medium, thereby resulting in the required final sample concentrations. Following green tea extract addition the plates were incubated for an additional 48 h at 37°C, 5 per cent CO_2, 95 per cent air and 100 per cent relative humidity. The medium without containing the samples were served as control and triplicate was maintained for all concentrations.

MTT Assay

3-[4,5-dimethylthiazol-2-yl] 2,5-diphenyltetrazolium bromide (MTT) is a yellow water soluble tetrazolium salt. A mitochondrial enzyme in living cells, succinate-dehydrogenase, cleaves the tetrazolium ring, converting the MTT to an insoluble purple formazan. Therefore, the amount of formazan produced is directly proportional to the number of viable cells. After 48 hours of incubation, 15µl of MTT (5mg/ml) in Phosphate Buffered Saline (PBS) was added to each well and incubated at 37°C for 4 hours. The medium with MTT was then flicked off and the formed formazan crystals were solubilized in 100µl of DMSO and the absorbance was measured at 570nm using micro plate reader. The per cent cell inhibition was determined using the following formula:

Per cent cell Inhibition = 100 – Abs (sample)/Abs (control) x 100.

Statistical Analysis

Nonlinear regression graph was plotted between per cent Cell inhibition and Log_{10} concentration and IC_{50} was determined using Graph Pad Prism software.

Chromosomal Aberration Studies using GTE in Peripheral Blood Lymphocytes of Cervical Cancer Patients

All data and samples were collected after obtaining approval from the Human Ethical Committee (ECR/209/Inst/TN/2013).

About 2.0 ml of venous blood from the experimental subject was drawn into a sterile heparinized syringe and 0.5 ml of the blood was inoculated under aseptic conditions into a culture vial containing 5.0 ml of culture medium, 1.0 ml of ab serum and 0.2 ml of phytohaemagglutinin (PHA). The cultures were incubated at 37°C for a period of 72 hrs and were shaken periodically twice a day in order to facilitate proper mixing of the medium and cells in culture. 48 h old cultures of lymphocytes were treated with 3ml of PFE individually and in binary mixtures and incubated for 24 hours. The dividing cells in 60ml of 0.4 mg/ml working solution were arrested at the metaphase stage by adding 0.05 ml of colchicine solution (0.01 per cent) at 30 minutes before harvesting the culture. 6 ml of pre-warmed hypotonic solution (0.075 m KCl) was added to the test tube after disturbing the pellet obtained after centrifugation at 1000 rpm for 20 minutes cells were further incubated for 7 minutes of 1 ml freshly prepared fixative (methanol and glacial acetic acid (3:1 v/v) was added and centrifuged at 1000 rpm for 10 minutes and two or three changes of the fixative were carried out to obtain a colourless cell pellet.

A test slide was prepared by placing a drop of the cell suspension on a clean chilled slide and dried immediately at 40°C for a few seconds on a hot plate. The slide was examined under a microscope to see whether the concentration of cells and the spread of the chromosomes enabled detailed examination of metaphases. The rest of the slides were prepared after making suitable dilutions of the cell suspension with fresh fixative. Fifty well spread metaphase plates of each subject were screened under oil immersion lens of the optical microscope and selected metaphases were photographed. The antigenotoxic efficacy of the pomegranate fruit extract was

determined by applying ANOVA and the association between total chromosomal alteration and cervical cancer risk was analysed using students 't' test.

Results

Cell Proliferation Assay (MTT) in HeLa Cells

In the present study, HeLa cells showed growth inhibition in a dose dependent manner when treated with green tea extract at concentrations ranging from 18.75µg – 300µg. The percentage of dead cells for each concentration was found to be 3.74, 16.05, 29.65, 67.68 and 79.40. The 50 per cent cytotoxic effect (IC_{50}) of green tea extract was found to be 111.9µg/ml (Table 20.1 and Figure 20.1).

Table 20.1: *In vitro* Cytotoxicity of MEGT on HeLa Cell Lines

Concentration (µg)	*Per cent Cell Inhibition*	*IC_{50}*	*R^2*
18.75	3.74	111.9 µg/ml	0.9824
37.5	16.05		
75	29.65		
150	67.68		
300	79.40		

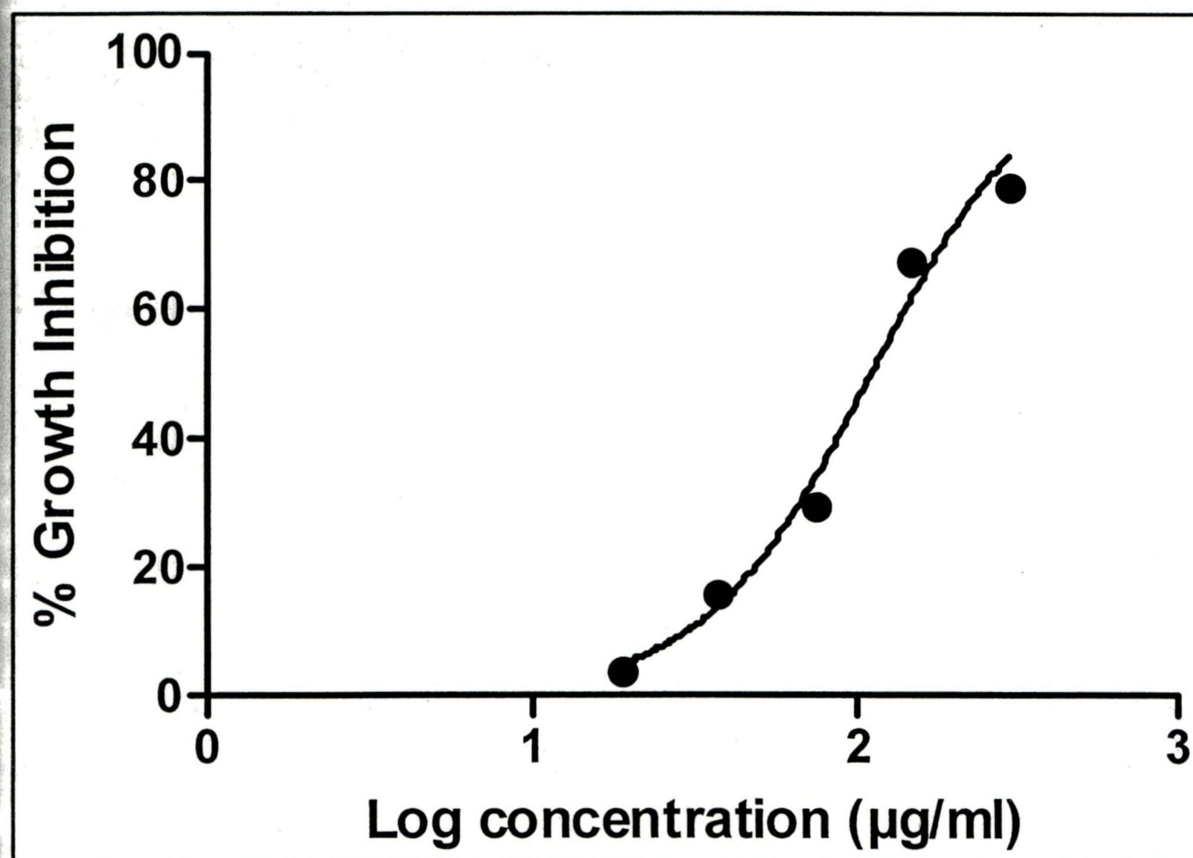

Figure 20.1: Percentage Growth Inhibition of MEGT against HeLa Cell Line.

Chromosomal Aberration Studies using Green Tea Extract in Peripheral Blood Lymphocytes of Cervical Cancer Patients

The result pertaining to the antigenotoxic potential of green tea extract in the cultured human lymphocytes of cervical cancer patients using chromosome aberration assay has been portrayed below. A total of ten blood samples were collected from cervical cancer patients. Samples treated with green tea extract were taken as experimental samples and untreated samples were considered as control group of samples. Table 20.2 depicts the profile of the experimental and control group of samples including age of the patients, stage and chromosomal alteration. Age of the patients ranged from 18 to 70 years. Samples collected were with different stages of disease (cervical cancer) namely stage I, stage II, stage III and stage IV. The total chromosomal alteration was found to be higher when the stage of cancer increased. The percentage inhibition of chromosomal alteration after the addition of methanol, chloroform, petroleum ether and water extracts of green tea was found to be 20 – [illegible] per cent.

Table 20.2: Chromosomal Aberration in Peripheral Blood Lymphocyte Culture Treated with Extract of Green Tea

Sl.No.	*Age*	*Stage*	*CA of Experimental Samples**		*Total CA of Experimental Samples**	*CA of Control Samples*		*Total CA of Control Samples*
			CTA	*CSA*		*CTA*	*CSA*	
1.	36	I	2	1	3	3	1	4
2.	43	III	1	3	4	4	0	4
3.	65	II	3	0	3	6	3	9
4.	18	I	1	0	1	3	2	5
5.	42	III	3	3	6	2	1	3
6.	39	I	2	1	3	3	3	6
7.	24	III	4	1	5	2	1	3
8.	48	II	1	0	1	7	5	12
9.	52	I	3	0	3	3	2	5
10.	68	II	1	1	2	2	3	5

* Cervical cancer samples treated with green tea (Experimental group of samples).

CTA: Chromatid Type of Aberration; CSA: Chromosome Type of Aberration; CA: Chromosomal Alteration; Stage: Stage of the disease.

Treatment with methanol extract inhibited chromosomal alteration to [illegible] ± 4.07 followed by treatment with chloroform extract to 4.2 ± 3.17. Treatment with petroleum ether extract showed poor inhibition (2.6 ± 0.89). Treatment with water extract showed no inhibition in the chromosomal alteration (Table 20.3). Of the four treatments, maximum reduction in chromosomal alteration was found in the methanol extract (p = 0.01).

Table 20.3: Frequency of Inhibition of Chromosomal Alteration in Human Lymphocytes of Cervical Cancer Patients Treated *in vitro* with Green Tea Extracts

Name of the Extracts	*Frequency of Inhibition (Per cent)*
Methanol	26.5 ± 4.07 (79.57 per cent)
Chloroform	4.2 ± 3.17 (12.6 per cent)
Petroleum ether	2.6 ± 0.89 (7.80 per cent)
Water	0 ± 0
F	1.64*
CD	7.38

*Significant at p = 0.01.

Table 20.4 illustrates the reduction in CTA, CSA and total CA in the experimental samples treated with methanol extract of green tea. Reduction in the number of CTA in the experimental samples was found to be 2.1 ± 1.10 than in the control samples which was found to be 3.5 ± 1.71. The results was found to be statistically significant at p = 0.05. Correspondingly CSA was found to reduce significantly in the experimental samples treated with methanolic extract (1 ± 1.15) than in control samples (2.1 ± 1.44). Alike CTA and CSA, the total chromosomal alteration (CA) was found to decrease in the experimental samples (3.1 ± 2.25) when compared to that of control samples (5.6 ± 3.15; p = 0.05).

Table 20.4: Effect of Methanol Extract of Green Tea in the Cultured Lymphocytes of Cervical Cancer Patients

	CTA	*CSA*	*Total CA*
Experimental samples	2.1 ± 1.10	1 ± 1.15	3.1 ± 2.25
Control samples	3.5 ± 1.71	2.1 ± 1.44	5.6 ± 3.15

** - Significant at 5 per cent level p = 0.05

Discussion

On a worldwide basis, cervical cancer is the second most prevalent cancer in women. India has a population of approximately 1.2 billion and accounts for a significant burden of cervical cancer. There is an estimated annual global incidence of 500,000 cases; in that India contributes 100,000 *i.e.* one fifth of the world burden (Shanta, 2003). Golikeri (2009) reported that there are an estimated 1.32 lakhs new cases and 74,000 deaths annually in India.

Plant derived extracts containing antioxidant principles such as flavonoids, phenolic compounds and tannins showed cytotoxicity towards tumor cells (Marklund *et al.*, 1982) and antitumor activity in experimental animals (Li and Oberley, 1997). Several of the green tea constituents have shown potent anticancer properties in many models based on the studies conducted throughout the world. Many of the beneficial properties of green tea were attributed to most abundant catechin, EGCG. But recent reports showed that green tea catechins have the potential to affect

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absorption and metabolism of ions because flavonoids interact with a variety of metal ions (Mira *et al.*, 2002).

The amount of catechins also varies in the original tea leaves due to differences in variety, origin and growing conditions (Khokhar and Magnudottir, 2002). Thus comparison of ingested doses in animal studies is not possible because the catechin quantification before administration is often not known. In this context, present study focuses on the crude methanolic extract of green tea for its *in vitro* cytotoxicity and genotoxic studies.

The discovery of new drugs and their development into commercial products takes place across the broad scope of the pharmaceutical industry and research institutes. The basic underpinning for this effort is the cumulative body of scientific and biomedical information generated worldwide in research institutes, academic centers, universities and industry (Kulkarni, 2007). It has been shown that, through several mechanisms, the anticarcinogenic potential of green tea catechins have correlated their cytotoxic effects with the induction of protein kinase, modulation of cell cycle regulations 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 (Gupta *et al.*, 2008).

It is currently used and applied in developmental stages of anticancer drug discovery where the most potent compounds are selected. Each and every new drug, cosmetics, additives, food and so on go through extensive, cytotoxicity testing before they are released to market for the use by people. This study involves large number of animal testing or experiments. There is much pressure, both human and economic, to perform at least a part of the cytotoxicity testing. The nature of the response must also be considered carefully. A toxic response *in-vitro* may be measured by changes in cell survival or metabolism while the major problem *in-vivo* may be a tissue response (*e.g.* an inflammatory reaction, fibrosis, kidney transport) or a systemic response (*e.g.* pyrexia, vascular dilation). For *in-vitro* testing to be more effective, models of these responses must be constructed, perhaps utilizing organotypic cultures resembled from several different cell types and maintained in the appropriate hormonal milieu.

It should not be assumed that complex tissues and even systemic reactions cannot be stimulated *in-vitro.* Assays for inflammatory responses, teratogenic disorders and neurological dysfunctions may be feasible *in vitro* given a proper understanding of cell-cell interaction. In addition to cancer research and virology, other areas come to depend heavily on tissue culture techniques (Freshney R, 2000). The *in vitro* cytotoxic potential was investigated as the ability of the plant extracts to inhibit tumour cell line growth. With this investigation we had focused on the ability of green tea extract against cervical cancer cell lines (HeLa). Several mechanisms of action were detected in HeLa cells.

After exposure of cells to green tea extract the cell line were treated with MTT Dye which results into the live cells converting the MTT to purple coloured formazan crystals, which are soluble in Dimethyl sulphoxide (DMSO). After solubilisation of

crystals absorption is taken on spectrophotometer at 570nm. With respect to the readings, graphs were plotted for per cent inhibition on Y-axis and concentration of drug on X-axis. The readings were directly converted into percentage. From the above graph IC_{50} value of green tea extract for HeLa cells was found to be 111.9µg/ml. The IC_{50} of extract on cell line less than 100µg/ml is categorized as a potential cytotoxic substance (Spavieri *et al.*, 2010). Hence present study shows the efficacy of MEGT against HeLa cells thereby suggesting protection against cervical cancer.

Sowjanya *et al.* (2009) reported that the chromosomal alteration analysis is one of the widely used parameters for testing the protection effects of natural compounds on the drug and chemical induced toxicity. The conceptual basis for using chromosomal aberrations in peripheral blood lymphocytes as a biomarker is the fact that extent of genetic damage in peripheral blood lymphocytes reflects similar events in the precursor cells for carcinogenic processes in the target tissues.

Boffetta *et al.* (2007), from their results of a cohort study from central Europe, provide support for the hypothesis that the occurrence of chromosomal aberrations in peripheral blood lymphocytes represent relevant events in carcinogenesis and may serve as a surrogate end point for cancer risk. They showed that a high frequency of chromosomal aberrations in peripheral blood lymphocytes, and in particular of chromosome type aberrations, is associated with increased risk of cancer. This hypothesis falls in line with the present study in that methanol extract of green tea extract decreased the number of total CA in the peripheral blood lymphocytes of cervical cancer patients. Gupta *et al.* (2009) studied the antigenotoxic effect of green tea extract against genotoxic damage induced by two anabolic steroids- Trenbolone and Methyltestosterone in cultured human lymphocytes, both in the absence and presence of metabolic activation. The results proved the antigenotoxic potential of green tea extract. Eroglu (2011) examined the cytogenetic effects of black tea and green tea in cultured peripheral blood lymphocytes and the results showed that black tea and green tea induced the mitotic and replication indexes and decreased micronuclei. He concluded that tea polyphenols protects the normal cells from genotoxic or carcinogenic agents, which indicated the therapeutic and antioxidative role of catechins, flavonoids or other tea compounds. Black tea and green tea showed considerable anticastogenic and antigenotoxic effects in human lymphocytes. Black tea and green tea could protect the normal cells from the genotoxic or carcinogenic agents (Eroglu, 2011). Many studies supported the antigenotoxic activity of green tea catechins. Kuroda (1996) and Isbrucker *et al.* (2006) demonstrated that tea catechins could suppress the genotoxic activity of various carcinogens with both *in vitro* and *in vivo*.

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