

Original Research Article

Anti-proliferative activity of green tea extract in Human Cervical Cancer Cells (HeLa)

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ABSTRACT

Keywords

Anti-proliferative effect;
Human cervical Cancer.

In this study antiproliferative effect of Green tea extract was evaluated on HeLa cell line. HeLa cells were cultured in DMEM medium and incubated with different concentrations (18.75, 37.5, 75, 150 and 300 $\mu\text{g/ml}$) of methanol extract of Green tea. Cell viability was assessed by MTT assay. Green tea decreased cell viability in malignant cells in a concentration dependent manner. The IC₅₀ value of green tea extract was found to be 111.9 $\mu\text{g/ml}$. It may be concluded that green tea could cause cell death in HeLa cells and can be considered as a promising antiproliferative agent against cervical carcinogenesis.

Introduction

Cancer of the cervix is the commonest genital tract malignancy in the female and it has been ranked second to breast cancer. About half a million new cases are seen worldwide each year, most occurring in developing countries (Ertem, 2009). The search for natural products which contains potential anticancer activity has begun recently. Over the past years number of approaches has been developed for clinical use and a number of anticancer drugs have been introduced. The main problems with these agents are the toxicity associated with them due to their lack of specificity, as these agents also kill healthy cells. Though a good number of anticancer agents have been developed from plants and their derived agents, development of a

safe, economic and site-specific anticancer drug is still a challenge.

The plant of tea (*Camellia sinensis*) has been grown in south east Asia for thousands of years and now is 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). 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. Although green tea has several beneficial effects on health, the effects of its constituents may be beneficial up to a certain dose yet higher doses may cause some unknown adverse effects. Moreover, the effects of green tea catechins may not be similar in all individuals. Schmidt *et al.* (2005) reported that EGCG of green tea extract is cytotoxic, and higher consumption of green tea can exert acute cytotoxicity in liver cells, a major metabolic organ in the body. Another study found that higher intake of green tea might cause oxidative DNA damage to hamster pancreas and liver (Takabayashi *et al.*, 2004) and clarified that EGCG acts as a pro-oxidant, rather than an antioxidant in pancreatic β cells *in vivo*.

In response to the above findings it is essential to find out the extent of toxicity of the green tea extract, its ability to kill the cells and discriminate property between replicating cells and non-replicating cells. Usually in oncology research and clinical practices, anti-proliferative assays are used in the assessment of cancer types of individual patients (Edmondson *et al.*, 1988 and Fotakis and Timbrell, 2006). Though animal models provide more predictable results, *in vitro* testing is preferred prior to *in vivo* testing of potential chemotherapeutic agents. *In vitro* cultures can be cultivated under a controlled environment (pH, temperature, humidity, oxygen / CO₂ balance etc.) resulting in a homogenous batches of cells and thus minimizing experimental errors. Methyl thiazolyldiphenyl-tetrazolium bromide (MTT) assay has been described as rapid, simple and reproducible method, widely

used in the screening of anticancer drugs and to measure the tumor cell proliferation. Hence in the current study, the antiproliferative properties of green tea extract were studied using this assay in HeLa cell lines.

Materials and Methods

Preparation of Leaf Extract

Camellia sinensis leaves were collected from tea estate, Valparai, Coimbatore and were air dried and grounded to fine powder.

Then the powdered material was extracted with methanol by using soxhlet apparatus. The solvent was removed by evaporation and extract was concentrated by using vacuum rotator evaporator.

Chemicals

3-(4, 5-dimethyl thiazol-2-yl)-5-diphenyl tetrazolium bromide (MTT), Fetal Bovine Serum (FBS), Phosphate Buffered Saline (PBS), Dulbecco's Modified Eagle's Medium (DMEM) and Trypsin were obtained from sigma Aldrich Co, St. Louis, USA. EDTA, glucose, Trichloroacetic acid (TCA), Acetic acid, Tris base and antibiotics from Hi-media Laboratories Ltd., Mumbai. Dimethyl Sulfoxide (DMSO) and Propanol from E. Merck Ltd., Mumbai, India.

Cell Lines and Culture Medium

The human cervical cancer cell line (He La) was obtained from National Centre for Cell Sciences (NCCS), Pune and grown in Eagles minimum essential medium (EMEM) containing 10% Fetal Bovine Serum (FBS). All cells were maintained at 37°C, 5% CO₂, 95% air and 100% relative

humidity. Maintenance cultures were passaged weekly, and the culture medium was changed twice a week.

Cell Treatment Procedure

The monolayer cells were detached with trypsin ethylenediamine tetra acetic acid (EDTA) to make single cell suspensions and viable cells were counted using a hemocytometer and diluted with medium containing 5% FBS to give final density of 1×10^5 cells / ml. One hundred microlitres per well of cell suspension were seeded in to 96-well plates at plating density of 10,000 cells / well and incubated to allow for cell attachment at 37°C, 5% CO₂, 95% air and 100% relative humidity. After 24 h the cells were treated with serial concentration of the test samples. 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 concentration. Aliquots of 100 µl of these different sample dilutions were added to the appropriate wells already containing 100 µl of medium, resulted the required final sample concentrations. Following the treatment with methanolic extract of green tea, the plates were incubated for an additional 48 h at 37°C 5% CO₂, 95% air and 100% relative humidity. The medium without samples were served as control and triplicate was maintained for all concentrations.

MTT Assay

3-[4, 5-dimethyl thiazol-2-yl] 2, 5-diphenyl tetra-zolium 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 product is directly proportional to the number of viable cells. After 48 h of incubation, 15 µl of MTT (5 µg / ml) in phosphate buffered saline (PBS) was added to each well and incubated at 37°C for 4 h. The medium with MTT was then flicked off and the formed formazan crystals were solubilized in 100 µl of DMSO and then measured the absorbance at 570 nm using microplate reader. The % cell inhibition was determined using the following formula: Percentage cell inhibition = $100 - \frac{\text{Abs (Sample)}}{\text{Abs (Control)}} \times 100$.

Statistical analysis

Non linear regression graph was plotted between % cell inhibition and log₁₀ concentration and IC₅₀ was determined using graph pad prism software.

Results and Discussion

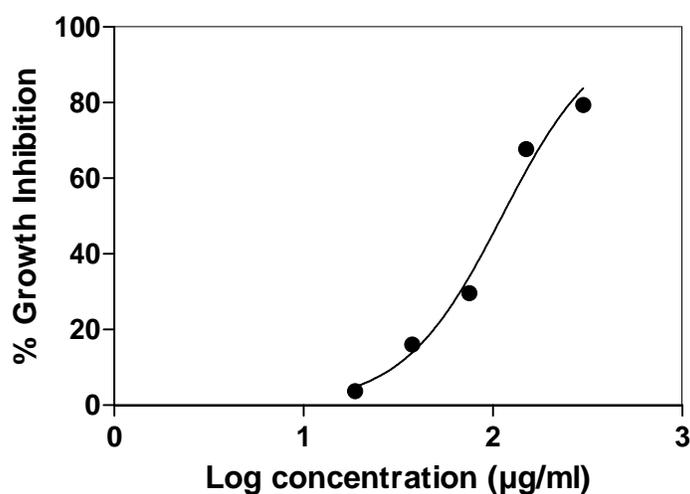
The results for cell growth inhibition by the extract against HeLa cell lines for various concentrations is shown in table 1. In the present study HeLa cells showed growth inhibition in a dose dependent manner when treated with green tea extract at concentrations ranging from 18.75 µg – 300 µg. The percentage of dead cells for each concentration was found to be 3.74, 16.05, 29.65, 67.68 and 79.40. The 50% cytotoxic effect (IC₅₀) of green tea extract was found to be 111.9 µg / ml (Table I and Figs. 1 & 2).

The utility of cell lines acquired from tumors allows the investigation of tumor cells in a simplified and controlled environment (Arya *et al.*, 2011). There are specific advantages and disadvantages to

Table.1 In vitro Cytotoxicity of MEGT on HeLa cell lines

Plant Extract	Concentration (µg / ml)	% inhibition	IC ₅₀ µg / ml	R ²
Green tea	18.75	3.74	111.9	0.9824
	37.5	16.05		
	75	29.65		
	150	67.68		
	300	79.40		

Fig.1 Percentage growth inhibition of MEGT against HeLa cell line



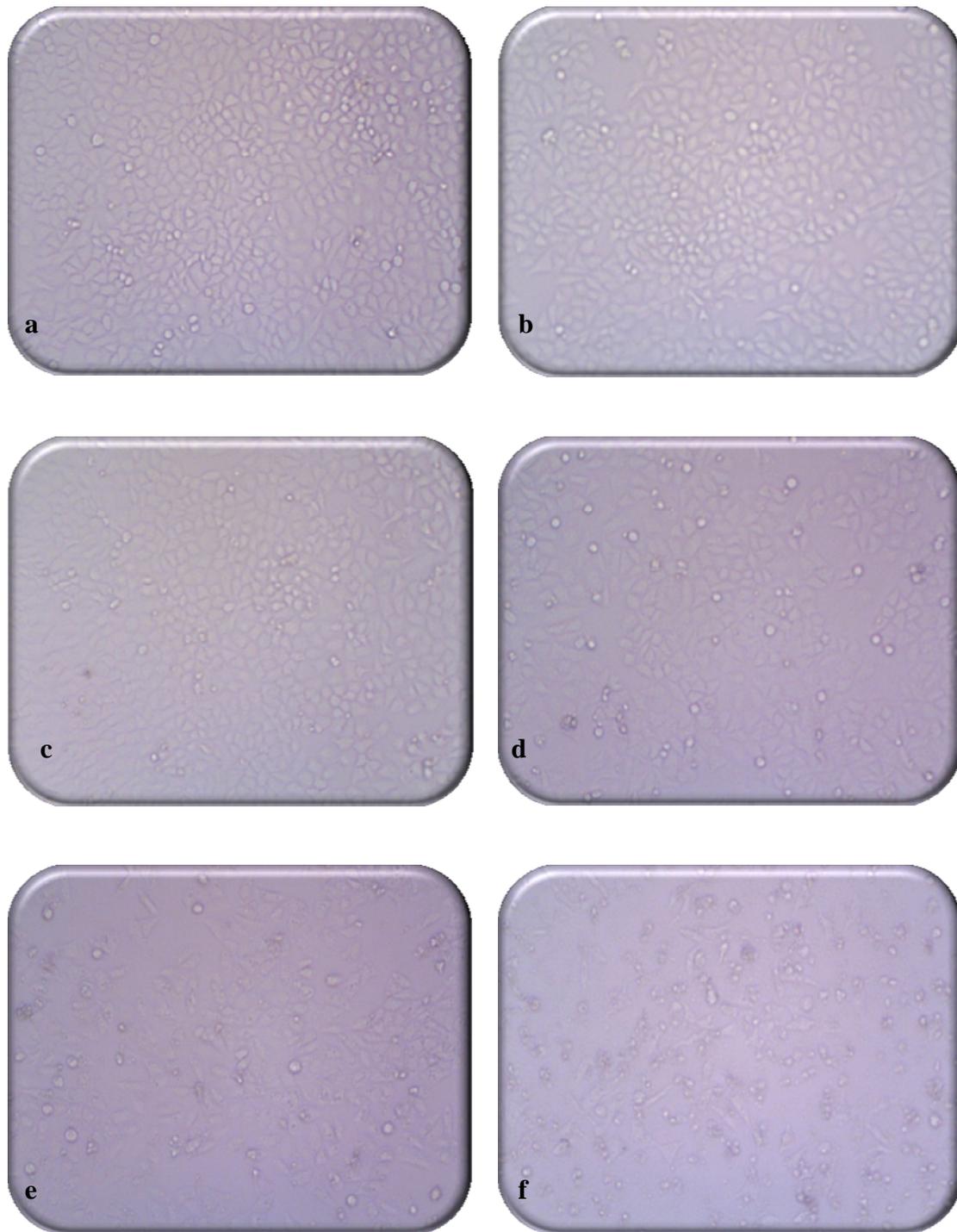
exploit cancer cell lines over animal models. These then dictate the nature of the experiment that can be organised. In the last few decades, studies with cell lines can serve as an initial screen for agents that might regulate drug resistance.

Green tea has been widely studied for its polyphenols because it has antioxidant activity. Now-a-days, after this antioxidant was found to offer protection against the occurrence of cancer, studies evaluated the effects of green tea extracts on different cell lines (Ledy *et al.*, 2012). In the present study the HeLa cell lines are used as a model for studying cervical cancer.

Several mechanisms of action were detected in HeLa cells. The IC₅₀ of extract on cell line less than 100 µg / ml is categorized as a potential cytotoxic substance (Spavieri *et al.*, 2010). In the present study, methanol extract of green tea was found to be moderately cytotoxic towards human HeLa in MTT assay and the concentration required for 50% cell death was found to be 111.9 µg / ml.

Hence present study shows the efficacy of MEGT for the antiproliferation of HeLa cells thus suggesting protection against cervical cancer.

Fig.2 Proliferation of HeLa cells treated with MEGT



a – Control ; b – 18.75 µg ; c – 37.5 µg ; d – 75 µg ; e – 150 µg ; f – 300 µg ;

In summary, the present study demonstrated that methanol extract of green tea is a potent anti-cancer compound with an IC₅₀ of 111.9 µg / ml inducing growth inhibition in the human cervical cancer cells. Further research based on animal models may resolve *in vivo* efficacy of green tea.

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