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## **Synergistic Effects of Green Tea Catechin and Phytic Acid Increases the Cytotoxic Effects on Human Colonic Adenocarcinoma Cell Lines**

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### **ABSTRACT**

Colorectal cancer is sensitive to dietary intervention. Epidemiological data suggest that high intake of fruits and vegetables may decrease colorectal cancer risk. This experiment was designed to study the preventive effect of green tea catechins and phytic acid in cell culture against the colon tumors. Selected concentrations (0.25 to 25 mM) of green tea catechins and phytic acid were used to treat Caco-2 and HT29 cells maintained in Dulbecco's modified Eagles Medium (DMEM) with 10% fetal bovine serum. For assay  $5 \times 10^5$  cells/well/100  $\mu$ L were seeded to a 24 well culture plate and incubated at 37°C and 7% CO<sub>2</sub> until monolayer was developed, then 400  $\mu$ L of fresh serum-free DMEM was added to the 24 wells. At the same time selected concentrations (0.25-25 mM) of green tea catechins and phytic acid made up to 100  $\mu$ L with saline was added and incubated for 24 and 48 h to determine the cytotoxic effect in term of Lactate Dehydrogenase (LDH), Alkaline Phosphatase (AP) and apoptosis assay. The results of this experiment showed that, when Caco-2 and HT29 cells were treated with various concentrations (0.25-25 mM) of green tea catechins and phytic acid for 24 and 48 h, the LDH and AP release was dose and time dependent. When HT29 cells treated with 25 mM concentration of green tea catechins for 24 h and combination of green tea catechins and phytic acid at 5 mM concentration for 12 h showed signs of membrane blebbing which indicates that singly and/or in combination of green tea catechins and phytic acid showed the anti-tumorigenesis activity. Thus, green tea catechins and phytic acid makes it promising chemo-preventive agents of colon cancer.

**Key words:** Green tea catechins, phytic acid, LDH, AP, Caco-2, HT29 cell

### **INTRODUCTION**

Cancer is the major public health problem in the western societies. In 2007, it causes estimate deaths of 7.6 million (about 20, 000 cancer deaths/day) worldwide (American Cancer Society, 2007). A total of 1,529,560 new cancer cases and 569,490 deaths from cancer corresponding to greater than 1500 deaths per day are projected to occur in the United States in 2010 (American Cancer Society, 2010). Cancer is a multifactor and multistage disease, whose incidence primarily depends on hereditary and environmental factors. Environmental factors are considered as modifiable factors which play a vital role in the incidence of cancer. Consumption of a low fiber diet, frequent exposure to certain chemicals, residence in industrialized areas and low physical activity are the environmental factors which play a vital role in the progression of cancer. Diet is the single most

important factor which contributes to 35-40% of the incidence of human cancer (Doll and Peto, 1981). Dietary factors are estimated to account for approximately 30% of cancers in western countries, making diet second only to tobacco as a preventable cause of cancer (WHO, 2010).

Several epidemiological studies have shown that there is an association between reduced risk of colon cancer and diets rich in fiber or vegetables (Steinmetz *et al.*, 1994; Greenward *et al.*, 1987; Willett, 1994; Wahab *et al.*, 2008). Consuming a diet rich in plant foods will provide a milieu of phytochemicals which are non-nutritive substances possess health-protective benefits (Liu, 2003; Kris-Etherton *et al.*, 2002). Whole grains, fruits, vegetables, nuts and seeds contain various phytochemicals and other natural antioxidants that have been associated with protection from various degenerative diseases. Health benefits resulting from the consumption of natural plant products rich in bioactive substances has promoted growing interest from pharmaceutical, food and cosmetic industries (Capecka *et al.*, 2005). Many epidemiological studies have shown that green tea may decrease the risk of various types of cancers (Kohlmeier *et al.*, 1997; Katiyar and Mukhtar, 1996; Yang and Wang, 1993). Most of the reports show positive cancer preventative effects in Asian countries, where tea is consumed as a main beverage. Some laboratories have shown that green tea catechins inhibit various forms of cancer in animal models. These include cancers of the skin, lung, stomach, colon, liver, esophagus bladder and breast (Katiyar and Mukhtar, 1996; Conney *et al.*, 1999). Studies have shown that green tea constituents possess various effects such as antioxidative (Matsuzaki and Hara, 1985), antihypercholesterolemic (Muramatsu *et al.*, 1986) antimutagenic (Kada *et al.*, 1985), antibiotic (Toda *et al.*, 1989), antihypertensive (Hara and Tonooka, 1990), anti-inflammatory (Sagesaka *et al.*, 1996) and antihyperglycemic (Shimizu *et al.*, 1988). The most important function of tea catechins is the antioxidant property due to their capacity to sequester metal ions and to scavenge reactive oxygen species (Weisburger, 1988). Phytic acid (Inositol hexa-phosphate) is found in cereal grains, nuts, and seeds. Anticarcinogenic effect of phytic acid has been shown both *in-vivo* and *in-vitro* (Shamsuddin *et al.*, 1988). Phytic acid is a strong chelating agent which forms chelates with metals. This principle is the basis for its anticarcinogenic effect (Graf and Eaton, 1985). Phytic acid has been shown to alter gene expression by affecting signal transduction pathway, cell cycle regulatory genes, and tumor suppressor genes (Graf *et al.*, 1987). Saied and Shamsuddin (1998) reported that phytic acid up-regulated the expression of tumor suppressor genes p53 and gene p21 waf1/Cip1. Verghese *et al.* (2006) reported that when Caco-2 cells were treated with various concentrations of phytic acid (0.25-40 mm), the LDH release ranged from 13.55-44.7% after 24 h of incubation.

Recent trends of identifying various compounds found in dietary sources in biological form or specific combinational form draw a huge attention among the researchers. Liu (2003) reported that natural phytochemicals present at low levels in fruits, vegetables, herbs and spices offer many health benefits but these compounds may not be effective or safe when consumed at higher doses. Liu (2004) suggested that additive and synergistic effects of phytochemicals in whole foods improve health benefits when compared to an isolated purified phytochemical extract or a dietary supplement which may be responsible for their anti-carcinogenic effects. Utilizing combinations of dietary ingredients may provide a promising alternative solution in controlling various degenerative diseases like cancer either by halting or inhibiting the diseases process. Understanding the influence of various bioactive compounds on molecular interactions and immuno-modulatory responses led to the emerging strategy of combinational chemoprevention (De Kok *et al.*, 2008). There is much unknown about the combinational effects of green tea and phytic acid and its potential benefits particularly with regard to colon carcinogenesis. Thus, this

experiment was designed to determine the cytotoxic effects of various concentrations (ranging from 0.25 to 25 mM) of green tea catechins and phytic acid singly and in combination on human colon cancer cell (Caco-2) and an adenocarcinoma cell (HT29).

## **MATERIALS AND METHODS**

**Cell culture:** Green tea catechins and phytic acid was purchased from Sigma Chemical Company (St. Louis, MO). The selected concentrations (ranging from 0.25 to 25 mM) of green tea catechins and phytic acid were prepared shortly before use. Caco-2 and HT29 human colon cancer cells were obtained from the American Type Cell Culture (ATCC, Manassas, VA). Cells were maintained in Dulbecco's modified Eagles Medium (DMEM) with 10% fetal bovine serum. For assay,  $5 \times 10^5$  cells/well/100  $\mu$ L were seeded to a 24 wells culture plate and incubated at 37°C and 7% CO<sub>2</sub> until monolayer was developed. Culture medium was replaced with fresh medium when medium appeared spent.

**Lactate dehydrogenase assay:** After the development of a monolayer, 400  $\mu$ L of fresh serum free DMEM was added to the 24 wells. At the same time selected concentrations (0.25-25 mM) of green tea catechins and phytic acid made up to 100  $\mu$ L with saline were added and incubated for 24 and 48 h. The experiment was carried out in replicates of 2 wells/concentration. After incubation, cells were centrifuged (300x g, 3 min) using a Beckman bench top centrifuge and cell free supernatant fluid (0.1 mL) was collected and LDH assay was conducted using a cytotoxic kit (Boehringer Mannheim, Anaheim, CA) according to Manufactures instructions. After 3 min of incubation at room temperature, the plates were read at 490/655 nm using a BIO-RAD (Hercules, CA) microplate reader. It is a colorimetric assay for quantification of cell death and cell lysis based on measurement of LDH release from damage cell. The cytotoxicity percentage was calculated by following formula:

$$\text{Cytotoxicity (\%)} = \frac{\text{Experimental valu} - \text{Low control}}{\text{High control} - \text{Low control}} \times 100$$

**Alkaline phosphatase activity:** Caco-2 and HT29 cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM) with 10% fetal bovine serum. For assay, 100  $\mu$ L of  $5 \times 10^5$  cells/well were seeded to a 24 wells culture plate and incubated at 37°C, 7% CO<sub>2</sub> until a monolayer was developed. After the development of a monolayer, 400  $\mu$ L of fresh serum free DMEM was added to the 24 wells. At the same time selected concentrations (0.25-25 mM) of green tea catechins and phytic acid made up to 100  $\mu$ L with saline was added and incubated for 24 and 48 h.

After incubation, cells were centrifuged (300x g, 3 min) and cell supernatant (0.1 mL) was placed in a 96-wells microtitre plate for AP assay. Alkaline phosphatase substrate buffer (100  $\mu$ L, 0.1 mol L<sup>-1</sup> tris, 0.1 mol L<sup>-1</sup> NaCl, 5 mmol L<sup>-1</sup> MgCl<sub>2</sub>, pH 9.5) containing p-nitrophenyl phosphate (PNPP, 1 mg mL<sup>-1</sup>) (Sigma Chemical, St. Louis, MO) was added to each well. After 3 min of incubation at room temperature, the plates were read at A105/593 nm using a Bio-Rad micro plate reader (Richmond, Ca).

**Apoptosis assay (microscopic):** Caco-2 and HT29 cells were grown on slide flaskets in the presence of 0.25-25 mM concentrations of green tea catechins and phytic acid and incubated for 12, 24 and 48 h and stained using Giemsa stain to determine any apoptotic activity.

**Statistical analysis:** Data were analyzed using SAS (2009). Mean differences were tested for statistical significance by ANOVA and means were separated using Tukey's studentized range test. Means differences were considered significant at  $p \leq 0.05$ .

## RESULTS

**Lactate dehydrogenase assay:** The cytotoxic effects of green tea catechins on human colonic adenocarcinoma cell lines (Caco-2 and HT29) following 24 and 48 h incubation was measured using the LDH and AP released assay. Cytotoxic effects were seen to be dose and time dependent. The release of LDH and AP was higher with higher concentrations of green tea catechins and longer incubation time for both cell lines (Caco-2 and HT29) compared to control. LDH release was (%) higher, when HT29 cells were treated with 5 and 25 mM green tea catechins compared to Low Control (LC) (cells without any treatment) after 24 and 48 h incubation (Fig. 1). Treatment with 5 mM green tea catechins resulted in over 60% of LDH release from HT29 cells after incubation for 24 and 48 h. When HT29 cells were treated with 0.25 and 1 mM of green tea catechins, LDH release was lower than 50% following 24 and 48 h incubation which was lower than incubation with 5 and 25 mM green tea catechins. LDH release was significantly ( $p < 0.05$ ) lower, when HT29 cells were treated with 0.25 mM green tea catechins compared to other treatment groups (1, 5, and 25 mM).

Caco-2 cells were treated with selected levels of green tea catechins for 24 and 48 h and LDH released (%) from the cytosol of damaged cells was measured (Fig. 1). When Caco-2 cells were incubated with 25 mM green tea catechins for 48 h, LDH release was higher than control (LC) and other treatment groups. When Caco-2 cell were incubated with 1 and 5 mM green tea catechins for 48 h and 25 mM for 24 h, LDH release was not different but LDH release (%) was higher than cells treated with 0.25, 1 and 5 mM green tea catechins for 24 h incubation. There were no differences in LDH release (%) from Caco-2 cells, when incubated with 0.25, 1 and 5 mM green tea catechins for 24 h (Fig. 1).

The cytotoxic effects of selected levels of phytic acid in human colonic adenocarcinoma cell lines (Caco-2 and HT29) are shown in Fig. 2. HT29 cells incubated with 25 mM phytic acid for 24 and

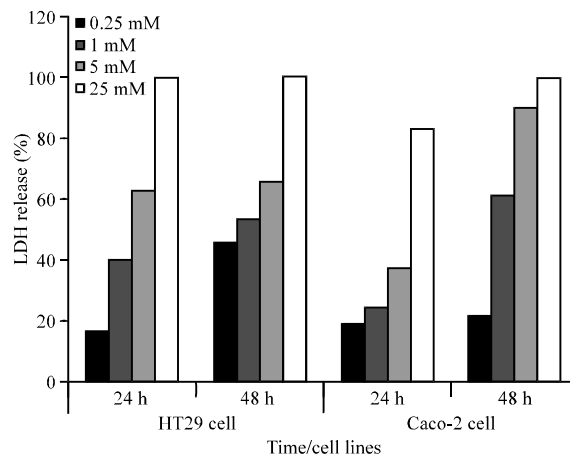


Fig. 1: Percent lactate dehydrogenase release after 24 and 48 h incubation of HT29 and Caco-2 cells by selected levels of green tea catechins

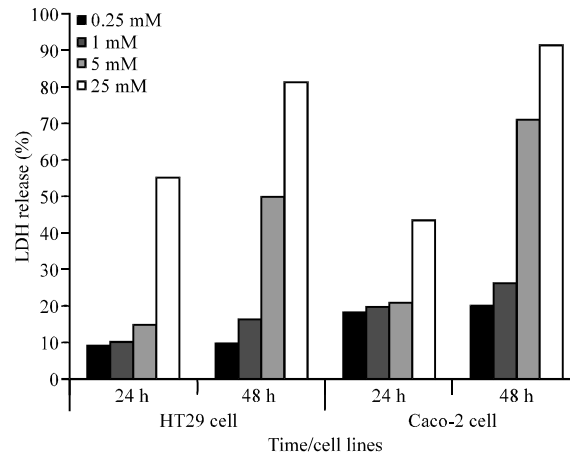


Fig. 2: Percent lactate dehydrogenase release after 24 and 48 h incubation of HT29 and Caco-2 cells by selected levels of phytic acid

48 h and 5 mM of phytic acid for 48 h, resulted in higher levels of LDH release (%) compared to control and other treatment groups (Fig. 2). HT29 cells incubated with 5 mM phytic acid for 48 h, resulted in less than 50% LDH release. When HT29 cells were incubated with 0.25, 1 and 5 mM phytic acid for 24 and 48 h, there was a lower percent (less than 20%) of LDH released from damaged cell (Fig. 2) except when cells were treated with 5 mM phytic acid for 48 h of incubation. The LDH release (%) was similar, when cells were incubated with 0.25, 1 and 5 mM phytic acid.

The percent LDH release was higher, when Caco-2 cells were treated with 25 mM phytic acid and incubated for 48 h compared to control and treatment groups (Fig. 2). A similar pattern was observed, when Caco-2 cells were incubated with 5 mM phytic acid for 48 h. The LDH released was lower (less than 30%), when Caco-2 cells were treated with 0.25 and 1 mM phytic acid for 24 and 48 h and 5 mM for 24 h of incubation (Fig. 2). When Caco-2 cells were treated with 25 mM phytic acid and incubated for 24 h, the LDH release was higher compared to those treated with 0.25, 1 and 5 mM phytic acid incubated for same period of time.

**Alkaline phosphatase activity:** AP release was higher (>75%), when HT29 cells were treated with 25 mM green tea catechins for 24 and 48 h compared to control (LC) and other treatment groups (Fig. 3). AP release from HT29 cells treated with 5 mM green tea catechins for 24 and 48 h was lower than that seen with 25 mM green tea catechins but higher than 1 and 0.25 mM green tea catechins incubated for 24 and 48 h (Fig. 3). When HT29 cells were treated with 0.25 and 1 mM green tea catechins for 24 h, AP release was lower compared to HT29 cells treated with 0.25 and 1 mM green tea catechins for 48 h (Fig. 3).

When Caco-2 cells were treated with 25 mM green tea catechins for 24 and 48 h, AP release (%) was higher compared to control and other treatment groups (Fig. 3). AP release (%) was lower, when Caco-2 cells were treated with 5 mM green tea catechins for 48 h compared to 25 mM green tea catechins for 24 and 48 h. When Caco-2 cells were treated with 0.25 and 1 mM green tea catechins for 48 h incubation and 5 mM of green tea catechins for 24 h, there were no differences in AP release (<30%) but there were differences when compared to 0.25 and 5 mM green tea catechins after 24 h incubation (Fig. 3). With increasing concentration of green tea catechins, there was higher amount of AP release from Caco-2 cells.

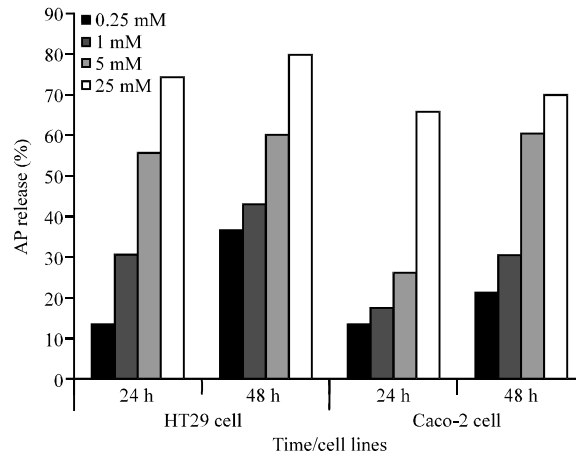


Fig. 3: Percent alkaline phosphatase release after 24 and 48 h incubation of HT29 and Caco-2 cells by selected levels of green tea catechins

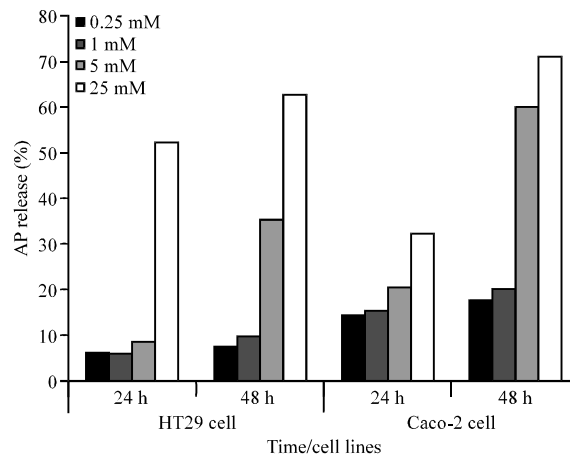


Fig. 4: Percent alkaline phosphatase release after 24 and 48 h incubation of HT29 and Caco-2 cells by selected levels of phytic acid

AP release from HT29 cells was higher, when treated with 25 mM phytic acid for 48 h (Fig. 4). AP release was lower, when HT29 cells were treated with 5 mM phytic acid for 48 h and 25 mM phytic acid for 24 h compared to 25 mM phytic acid after 48 h of incubation (Fig. 4). There were no differences in AP release, when HT29 cells were treated with 5 mM phytic acid for 24 h and 0.25 and 1 mM for 24 and 48 h (Fig. 4).

AP release was higher (75%) compared to the control (LC) and other treatment groups, when Caco-2 cells were incubated with 25 mM phytic acid for 48 h (Fig. 4). Incubation with 25 mM phytic acid for 24 h resulted in lower AP release from Caco-2 cells. AP release was lower from Caco-2 cells treated with 0.25, 1 mM phytic acid for 24 and 48 h and 5 mM phytic acid for 24 h compared to other treatment groups (5 mM for 48 h and 25 mM for 24 and 48 h) (Fig. 4).

### **Combinations of green tea catechins and phytic acid**

**Lactate dehydrogenase assay:** Based on the results of the first part of this experiment, cytotoxic effects of green tea catechins and phytic acid were determined using only three levels

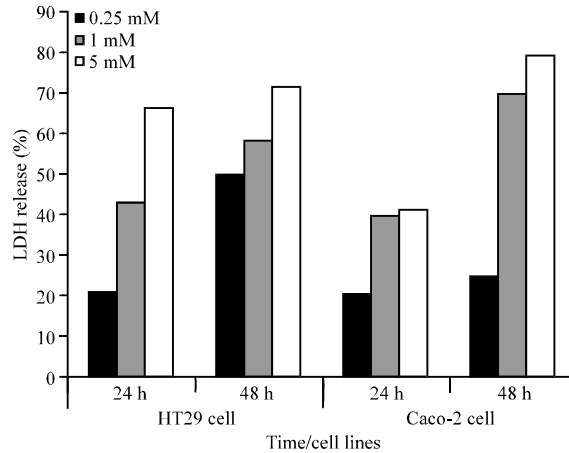


Fig. 5: Percent lactate dehydrogenase release after 24 and 48 h incubation of HT29 and Caco-2 cells by selected levels of green tea catechins and phytic acid

(0.25, 1 and 5 mM) in combination on HT29 and Caco-2 cell lines. The cytotoxic effects of selected levels of phytic acid and green tea catechins in combination on LDH release from HT29 cells are given in Fig. 5. Higher levels of LDH was release (>60%) from HT29 cells treated with combinations of green tea catechins and phytic acid (1 mM for 48 h and 5 mM for 24 and 48 h incubation, Fig. 5). When HT29 cells were treated with combinations of green tea catechins and phytic acid (1 mM for 24 h and 0.25 mM for 48 h), the LDH release was higher than 0.25 mM of the same combination after 24 h (Fig. 5). LDH release was significantly ( $p<0.05$ ) lower when HT29 cells were incubated with a combination of green tea catechins and phytic acid at 0.25 mM for 24 h compared to other treatment groups (Fig. 5).

There were higher amounts of LDH released from Caco-2 cells incubated with 1 and 5 mM of green tea catechins and phytic acid for 48 h (Fig. 5). LDH release was similar when Caco-2 cells were treated with combinations (1 and 5 mM) of green tea catechins and phytic acid and incubated for 24 h (Fig. 5). LDH release was lower compared to other treatment groups, when Caco-2 cells were treated and incubated with combinations of green tea catechins and phytic acid at 0.25 mM for 24 and 48 h (Fig. 5).

**Alkaline phosphatase activity:** AP release was higher (>50%) from HT29 cells treated with combinations of green tea catechins and phytic acid at 1 mM for 48 h and 5 mM for 24 h and 48 h compared to control (Fig. 6). When HT29 cells were treated with combinations of green tea catechins and phytic acid at 0.25 mM for 48 h and 1 mM for 24 h, AP release was higher compared to 0.25 mM of the same combination treated for 24 h (Fig. 6). AP release was lower than other treatment groups, when HT29 cells were treated with a combination of green tea catechins and phytic acid at 0.25 mM for 24 h.

There was a higher AP release from Caco-2 cell treated with a combination of green tea catechins and phytic acid (5 mM) after 48 h of incubation (Fig. 6). AP release was similar, when Caco-2 cells were treated with combinations of green tea catechins and phytic acid (5 mM) for 24 h and 1 mM for 48 h. AP release was significantly ( $p<0.05$ ) lower than other treatment groups, when Caco-2 cells were treated with combinations of green tea catechins and phytic acid at 0.25 mM for 24 and 48 h incubation and 1 mM for 24 h incubation.



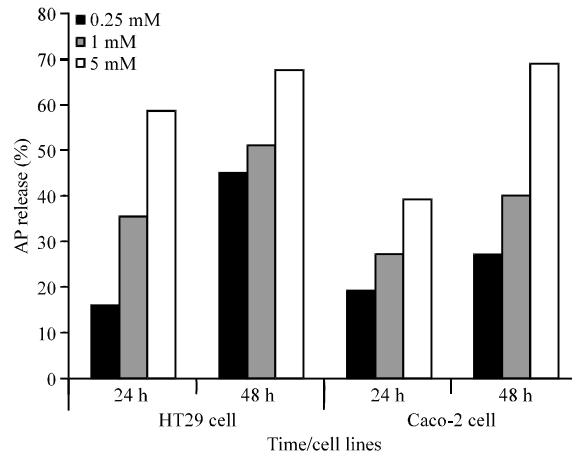


Fig. 6: Percent alkaline phosphatase release after 24 and 48 h incubation of HT29 and Caco-2 cells by selected levels of green tea catechins and phytic acid



Fig. 7: HT29 cells treated with 25 mM concentration of green tea catechins for 24 h of incubation showed the sign of membrane blebbing (Zeiosis)

**Microscopic apoptosis assay:** HT29 and Caco-2 cells were grown on slide flaskets in the presence of 0.25-25 mM green tea catechins and phytic acid and incubated for 12, 24 and 48 h to determine the morphological changes due to apoptotic activity induced by green tea catechins and phytic acid. HT29 cells treated with 25 mM green tea catechins for 24 h showed signs of membrane blebbing (Zeiosis) (Fig. 7). When HT29 cells were treated with 5 mM combinations of green tea catechins and phytic acid for 12 h, cells showed signs of membrane blebbing and cell leakage (Fig. 8). When Caco-2 cells were treated with various concentrations of green tea catechins and phytic acid for 24 and 48 h, signs of apoptosis were not observed.

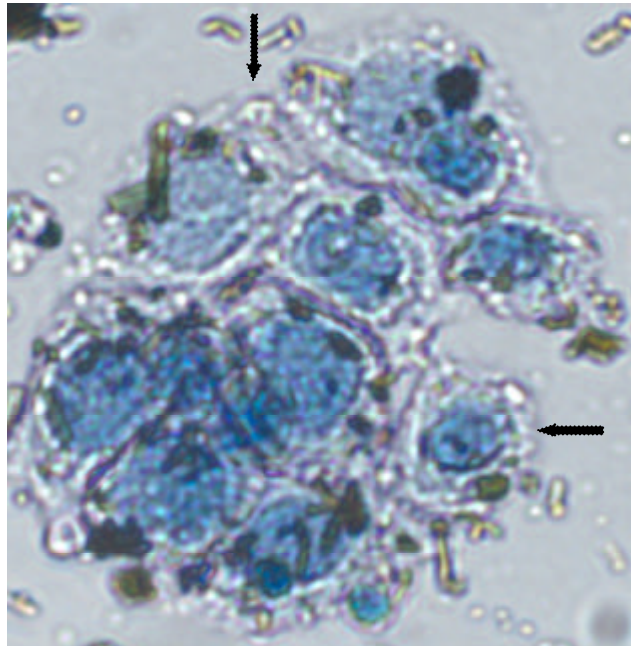


Fig. 8: HT29 cells treated with combinations of green tea catechins and phytic acid at 5 mM concentration for 12 h showed membrane blebbing and cell leakage

## DISCUSSION

Effects of green tea catechins and phytic acid singly and in combination in cell culture, using HT29 and Caco-2 cell were determined. Results of the cytotoxicity assays indicate that there are differences between the two cell lines concerning their sensitivity to green tea catechins and phytic acid. Caco-2 cells appear to be more sensitive as indicated by the LDH and AP released. In our opinion, this difference could be due to different uptake mechanisms of green tea catechins and phytic acid by the two cell lines. Various mechanisms have been proposed for the cytotoxicity effect of these phytochemicals.

In *in vitro* studies have indicated that phytic acid inhibits the growth of human breast (Shamsuddin and Vucenik, 1999), colon (Saied and Shamsuddin, 1998), prostate (Shamsuddin and Yang, 1995; Singh *et al.*, 2003), liver cancer cells as well as of rhabdomyosarcoma (Vucenik *et al.*, 1998a) and erythroleukemia cells (Shamsuddin *et al.*, 1992). Reports also showed that phytic acid inhibits the cell transformation in mouse epidermal JB6 cells (Huang *et al.*, 1997) and reverses the transformed phenotype of HepG2 liver cancer cells (Vucenik *et al.*, 1998b). It has been shown that exogenous administration of 1% IP6 in drinking water 1 week before or 2 weeks after the administration of azoxymethane inhibits the development of large intestinal cancer in F344 rats (Shamsuddin *et al.*, 1988). Phytic acid has been found to cause G1 cell cycle arrest in mammary cancer cell lines MCF-7 and MDA-MB 231 and in HT-29, a human colon cancer cell line (El-Sherbiny *et al.*, 2001). Phytic acid may exert its greatest biological effect through its antioxidant properties. It has been proposed that it forms an iron chelate which inhibits iron mediated oxidative reactions and limiting site specific DNA damage (Graf and Eaton, 1990). Graf and Eaton (1985) proposed that phytic acid's antioxidative properties help explain the suppression of colon

carcinogenesis by diets rich in phytic acid. Urbano *et al.* (2000) suggested that tumor progression may also be limited by phytic acid's chelation of other divalent cations (magnesium and zinc) since both are critical for tumor cell proliferation. Challa *et al.* (1997) reported that phytic acid may reduce the risk of cancer by inducing the phase II enzymes mainly GST which is responsible for detoxification of various carcinogens.

Cholewa *et al.* (2008) reported that inositol hexaphosphate enhanced TNFRI and decreased TNF- $\alpha$  and TNFRII transcription in Caco-2 cells stimulated for 12 h with inositol hexaphosphate seems to be the presumptive evidence for anti-inflammatory and anti-tumor activity of inositol hexaphosphate. The results of this experiment showed that, when HT29 and Caco-2 cells were treated with various concentrations (0.25-25 mM) of phytic acid for 24 and 48 h, LDH and AP release was dose and time dependent. In HT29 cells, the LDH release ranged from 9.11 to 10.24 and 55.27 to 81.36%, when cells were treated with 0.25 and 25 mM concentrations of phytic acid for 24 and 48 h, respectively. Where as in Caco-2 cells, LDH release ranged from 18.05 to 19.77 and 43.05 to 91.52%, when cells were treated with 0.25 and 25 mM phytic acid for 24 and 48 h of incubation, respectively. Alkaline Phosphatase (AP) release in HT29 cells was lower than LDH release and ranged from 6.33 to 7.83% and 52.37 to 62.91%, when cells were treated with 0.25 and 25 mM phytic acid for 24 and 48 h, respectively. This may be due to the fact that LDH in the cytosol is free which facilitates immediate release compared to AP which is associated with plasma membrane. Shamsuddin and Yang (1995) reported similar observations in MCF-7 and MDA-MB-231 human carcinoma cells lines. Verghese *et al.* (2006) reported that when Caco-2 cells were treated with various concentrations of phytic acid (0.25-40 mM), the LDH release ranged from 13.55-44.7% after 24 h of incubation.

Green tea catechins have gained significant acceptance as a cancer preventive agents and one of the important features of catechins is their interactions with various target molecules. Green tea catechins inhibited free radical formation and lipid peroxidation as well as induced phase II glutathione s-transferase and quinone reductase enzyme activity (Khan *et al.*, 1992). Katiyar *et al.* (1992) reported that green tea compounds inhibited cyclooxygenase and lipoxygenase activities. These activities of green tea catechins are responsible for their anticarcinogenic effects in *in vivo* and *in vitro* models Chen *et al.* (2002) reported that 100  $\mu$ M of EGCG released 9% LDH from Hepatic Stellate Cells (HSC). Data from our experiment indicated that LDH and AP release was dose and time dependent, when HT29 and Caco-2 cells were treated with green tea catechin. Chen *et al.* (2005) reported that, in a murine bone marrow mesenchymal stem cell line, LDH increased after 48 h of EGCG treatment. AP activity was also significantly augmented upon EGCG treatment for 4 days (Chen *et al.*, 2005).

Yang *et al.* (2007) investigated the effect of the two key constituents of green tea, epigallocatechin gallate and caffeine, on intestinal tumorigenesis in the ApcMin/+mouse model. They found that administration of epigallocatechin gallate at doses of 0.08% or 0.16% in drinking fluid significantly decreased small intestinal tumor formation by 37% or 47%. Hwang *et al.* (2007) reported that green tea derived Epigallocatechin-3-gallate exhibited a variety of molecular events in HT29 colon cancer cells including inhibition of cell growth, induction of apoptosis and ROS generation and inactivation of COX-2 expression and AMPK activation.

Suppression of apoptosis may lead to an increased cell lifespan or to an accumulation of genetic damage, ultimately leading to the process of developing degenerative diseases or process of

Carcinogenesis. In this experiment, when HT29 cells treated with 25 mM of green tea catechins for 24 h, they showed signs of membrane blebbing (Fig. 7). When HT29 cells were treated with 5 mM combinations of green tea catechins and phytic acid for 12 h, cells also showed signs of membrane blebbing and cell leakage (Fig. 8). Blebbing of plasma membrane and formation of apoptotic bodies are critical apoptotic events, indicating imminent cell death. Several reports have showed that plasma membrane blebbing is the most characteristic feature of apoptotic cells (Verghese *et al.*, 2006; Gupta *et al.*, 2003; Hastak *et al.*, 2003). Verghese *et al.* (2006) reported that Caco-2 cells treated with phytic acid showed signs of cell membrane blebbing which indicates that phytic acid might exert anticarcinogenic activity through induction of apoptosis. Results of this experiment also support this finding. Ahmad *et al.* (1997) reported that EGCG induced apoptosis and cell cycle arrest in cancer cells without affecting normal cells.

Findings of this experiment showed that higher percent of LDH and AP release, membrane blebbing in Caco-2 and HT29 cells incubated with combinations of green tea catechins and phytic acid rather than singly, also showed that green tea catechins and phytic acid exert strong anticarcinogenic effect by induction of apoptosis in cancer cells. Further studies on the effects of dietary green tea catechins and phytic acid as well as other potential anticarcinogenic agents must include a careful evaluation of the influence of these chemicals on metabolic activities. A clear understanding of molecular mechanisms of cancer prevention involved is needed for use in chemopreventive strategies. Therefore, identification of more molecular targets and biomarkers for green tea catechins and phytic acid is essential for improving the design of clinical trials and will greatly assist in better understanding of the mechanisms underlying its anti-cancer activity.

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