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ANTICANCER ACTIVITY OF GALLIC ACID ON CANCER CELL LINES, HCT15 AND MDA MB 231

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ABSTRACT

Cancer is the main leading cause of cancer deaths worldwide and chemotherapy is mainly used to treat cancer. However, the severe side effects of the drugs led the researchers to search for an alternative. Gallic acid, being a polyphenols, has been reported for its antiproliferative activity against many cancer cell lines. Moreover, its cytoprotective activity made gallic acid a potential compound in cancer therapy. Since colon cancer and breast cancers are among the most prevalent, the present study examines the anticancer property of gallic acid against two these two cancers, HCT15, human colon cancer cell line and MDA MB 231, human breast cancer cell line. The finding estimated the IC_{50} of the compound against the two cell lines. The present study also predicted the possible mechanism of the activity to be apoptosis, yet a detailed study is needed to find out the molecular targets.

KEYWORDS: Anticancer, Gallic Acid, HCT15, MDA MB231, MTT Assay

INTRODUCTION

Cancer is one of the major concerns around the world (Parasramka and Gupta, 2012), as it is one of the leading causes of death worldwide (Berghe, 2012). It is a group of disease where it affects all living cells, at all ages and in both genders (Nair, *et. al.*, 2005). In 2008, about 12.7 million cases and 7.6 million deaths were caused due to cancer, (Jemal *et. al.*, 2011) this has dramatically increased recently. According to The International Agency for Research on Cancer (IARC), WHO, the latest world cancer statistics estimated 14.1million new cancer cases and 8.2 million cancer deaths in 2012. Lung, breast and colorectum cancers were identified as most common occurrences worldwide (Press release, 2013). The prevalence of colon cancer is mainly associated with age.

It is estimated that about 90% of the colon cancer occurs after the age of 50 (American cancer). Moreover, breast cancer is also one of the prevalent cancers which occurs at a high frequency and is the cause of death in women in majority of cases. At present, surgery, chemotherapy and radiotherapy are the main conventional methods being used in the treatment of cancer. In modern medicine, chemotherapy is used most extensively. (Ravikanth, *et. al.*, 2001; Love, *et. al.*, 1989). Although the drugs show their efficacy, their side effects are very severe. The common side effects with anticancer drugs are nausea, hair loss, and tiredness (Yoon and Lui, 2007; Rates 2001). Hence, polyphenols are considered as an alternative due to its anticancer properties (Ramos, 2008).

Gallic acid is such a polyphenolic compound with reported anticancer properties on different cancers. It is also well known for its protective activity on normal cells which made gallic acid as a pivotal compound for cancer therapy (Li, *et. al.*, 2010; Verma, *et. al.*, 2013). The present study targets to examine the anticancer property of gallic acid

against the colon cancer and breast cancer cell lines. The impact of the study will pave a way to develop gallic acid as a significant therapeutic agent to treat and prevent cancer.

MATERIALS AND METHODS

Cell Culture and Reagent

Human colon cancer cell line, HCT15 and breast cancer cell line, MDA MB 231 were procured from National Centre for Cell Science (NCCS), Pune, India. The cell lines were grown in RPMI1640 and DMEM respectively, which were supplemented with 10% serum, penicillin (100IU/ml) and streptomycin (100µg/ml). Cell culture related chemicals were from HiMedia (India). All the other reagents were procured from Sigma Aldrich (USA).

Treatment

Sterile filtered gallic acid solution in water was stored at -20^{0}C as aliquots for further use. The cells were seeded in 6-well plate at a density 3 X 10^5 cells/well and incubated for one day at 37^{0}C, 5% CO_2 and 95% humidity. The following day, the cells were exposed to varying concentrations (0, 5, 10, 25, 50 and 100 µg/ml) of gallic acid for 24hr at 37^{0}C, 5% CO_2 and 95% humidity.

Morphological Analysis and MTT Assay

The untreated and treated cells were observed under phase contrast microscope at 100X magnification for any changes in the cell morphology. Further, the cells were taken for MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide) assay (Mosmann, 1983) with minor modification. MTT assay serves as indication of viable cells with intact mitochondria. The formazan crystals formed by the dehydroggenase enzymes give colorimetric reading. The cells washed with PBS (phosphate buffer saline) were incubated with MTT (100µg/ml) in dark at 37^{0}C for 5 hr. DMSO (dimethyl sulfoxide) was used to dissolve the formazan crystals and O. D. was read at 540nm. The absorbance of the untreated cells was considered as 100% viable cells. The percent viable cell was plotted on Y axis and concentration on X axis.

RESULTS AND DISCUSSIONS

Colon cancer and breast cancer are the main leading cause of cancer deaths worldwide. Thus, the present preliminary studies were done on human colon cancer cell line HCT15 and breast cancer cell line MDA MB 231 for the anticancer property of gallic acid. Gallic acid is present in green tea, fruits and vegetables (Faried, *et. al.*, 2007). The anticancer activity of gallic acid was found to be dose dependent in both the cell lines. The morphological analysis from the photomicrograph depicted the morphological changes in the treated cells like cell shrinkage, rounding of cells and detachment from the substratum (Figure 1). These changes are considered to be hallmarks of the cells undergoing apoptosis (Saraste and Pulkki, 2000). The cell viability was tested by MTT assay. It is one of the gold standard protocols to assess cytotoxicity due to its rapidity and precision (Mosmann, 1983). The percentage cell viability was calculated by multiplying the ratio of O. D. of treated cells and O. D. of untreated cells by 100. The IC_{50} of the compound was found to be 96 µg/ml and 80 µg/ml on HCT15 and MDA MB 231 respectively (Figure 2). Hence the present investigation exhibits gallic acid as a potential anticancer agent as many studies have also been reported on its antiproliferative property on different cancers like cervical cancer (Zhao and Hu, 2013), stomach cancer, prostate cancer, lung cancer and many other cancers (Verma, *et. al.*, 2013). However, a detailed study is required to understand the molecular mechanism of its activity.

CONCLUSIONS

The present study illustrated the anticancer activity of gallic acid on two of the most prevalent cancers, colon cancer and breast cancer. Furthermore, the treated cells demonstrated morphological changes, reduction in cell number, cell shrinkage, detachment from substratum, condensation and fragmentation of chromatin and presence of apoptotic bodies. These evidences revealed the signs of apoptosis in the treated cells. Henceforth, further molecular studies have to be done to confirm the molecular pathways responsible for cell death.

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APPENDICES

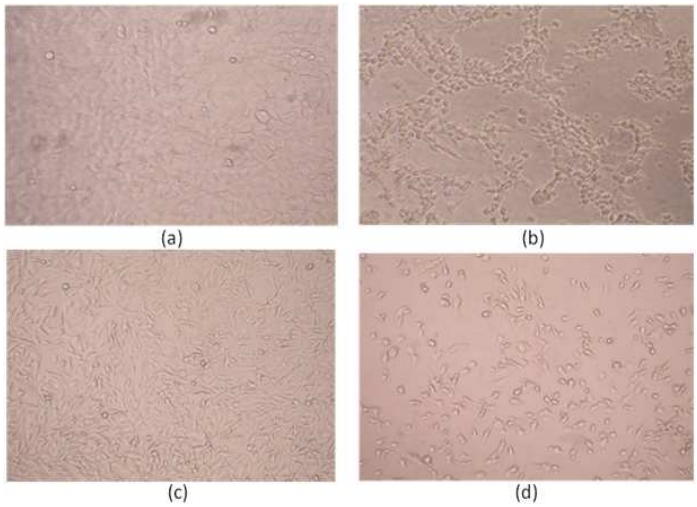

Figure 1: Photomicrographs of the Cell Lines Showing the Morphological Changes (a) Untreated HCT15 Cells, (b) HCT15 Cells Treated with 96 µg/Ml of Gallic Acid, (c) Untreated MDA MB 231 Cells & (d) MDA MB 231 Cells Treated with 80 µg/Ml of Gallic Acid. Cells Were Visualized under Phase Contrast Microscope (Magnification 100X)

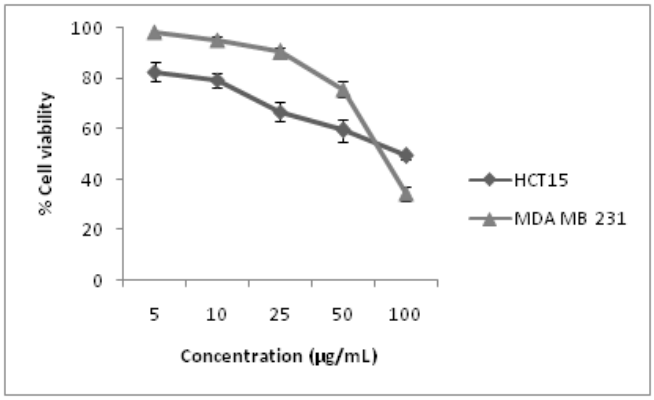

Figure 2: Cytotoxic Activity of Gallic Acid on HCT15 and MDA MB 231 Cell Lines. Values are Expressed as Mean +/- SD (N=5) Percentage Cell Viability