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Anti-Cancer Therapy: Chlorogenic Acid, Gallic Acid and Ellagic Acid in Synergism

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Abstract : *Phytochemicals have been used as effective agents for treating illnesses. Its use has been widely increased in the present scenario where commercial drugs are carrying a huge burden of side effects. Many phytochemicals have potent anticancer properties. The present study was undertaken to study the synergistic effect of anticancer phytochemicals Chlorogenic acid, Gallic acid and Ellagic acid on MDA MB 231 Breast cancer cells. The study was evaluated using cytotoxic assay such as MTT assay and migration assay via scratch well assay. The results demonstrate that the selected phytochemicals are highly cytotoxic to the cells and thus, synergistic studies were performed and the most cytotoxic combinations were proceeded with further assay which reveals that there is no migration observed in the presence of selected phytochemical combinations, thus revealing that treatment must be anti-metastatic.*

Keywords : *Chlorogenic acid, Ellagic acid, Gallic acid, MDA MB 231, MTT Assay, Scratch Assay.*

I. Introduction

Chlorogenic acid (CGA), the ester of caffeic acid with quinic acid, is one of the most abundant polyphenols in the human diet with coffee, fruits and vegetables as its major sources [1]. Peaches and plums contains higher amount of chlorogenic acid which is capable of killing cancerous cells [2]. CGA inhibits cell growth, regulates cell cycle, and induces apoptosis pathways [3].

Gallic acid (GA) or 3,4,5-trihydroxybenzoic acid is a natural antioxidant present in green tea, grapes, strawberries, bananas, gallnuts, sumac, witch hazel and many other fruits [4]. GA up-regulates the proapoptosis protein and down-regulates anti apoptosis proteins [5].

Ellagic acid (EA) is a polyphenolic compound present in fruits and berries such as pomegranates, walnuts, strawberries, blackberries, raspberries, oak acorns and oak aged red wine. It has anti-carcinogenic, anti-oxidant and anti-fibrosis properties [6, 7, 8, 9].

Breast cancer is a malignant (cancerous) growth that begins in the ducts and lobes of the breast. We hypothesize that these phytochemicals may kill breast cancer cells MDA MB 231 in a synergistic manner. The present study deals with investigation of the effect of Chlorogenic acid, Gallic acid and Ellagic acid on MDA MB 231 breast cancer cell line.

II. Materials And Methods

2.1 Maintenance of MDA MB 231 Cell Line

MDA-MB-231 breast cancer cells were obtained from NCCS, Pune, India. The cell line was maintained and propagated in Dulbecco's Modified Eagle's Medium (DMEM) containing 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin and were cultured up to ~90% confluence as adherent monolayer and maintained in a CO_2 incubator with 5% CO_2 at 37°C in a humidified atmosphere throughout the study. After confluence was attained, the cells were trypsinized and passaged as and when required.

2.2 Cell Viability Assay

This assay includes growing of 10,000 cells per well in a 96-well flat bottom cultured plates and measuring the cytotoxicity using a yellow coloured 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) dye which gets reduced by mitochondrial succinate dehydrogenase into insoluble purple coloured formazan crystals. All cultures were treated with selected phytochemicals for 4 days with successive incubation of 24 hours at 37°C in a humidified CO_2 incubator. After 4 days, 25µl of MTT (5mg/ml in PBS) was added to each well, and the plate was incubated for further 4 h at 37°C. The resulting formazan crystals were dissolved in 200µl DMSO and 25µl glycine buffer with gentle shaking at 37°C, and absorbance was measured at 570nm with an ELISA reader [10].

2.3 Cell Migration Assay

In this assay, cells were cultured ($1x10^6$) in a 6-well (60 mm) plate to allow them to adhere and to obtain confluent monolayer. After 24 hours, when confluency was attained, a scratch was made in the monolayer with the help of a p200 pipette tip. Then, each plate was treated with a different combination of phytochemical for consecutive 3 days at a particular time period. The effect of phytochemicals on migration of cells in the scratch was recorded and compared with the media control [11].

2.4 Statistical Analysis

All the data was analysed using Analyse It Software. One-way ANOVA was performed and p value <0.001 were considered as significant.

III. Results And Discussion

3.1 Cell Viability Assay

Various concentrations of selected phytochemicals viz., Chlorogenic acid (CGA) ranging from 150-500 µM/100µL [12], Gallic acid (GA) ranging from 5-100 µg/mL [13], and Ellagic acid (EA) ranging from 10-100 µM [14] were used against media control to study the viability of MDA MB 231 using MTT assay. Cyclophosphamide (CYCLO) (5-25 µg/100µL) was used as a standard drug for comparing the effects of selected phytochemical doses. Interestingly, Chlorogenic acid, Gallic acid, Ellagic acid and Cyclophosphamide showed dose dependent inhibition. From this, the best cytotoxic doses were selected for further studies. This includes Chlorogenic acid (150 µM/100µL) showing 12.194% viability, Gallic acid (5 µg/mL) showing 16.013252% viability, Ellagic acid (100 µM) showing 30.60004% viability and Cyclophosphamide (5 µg/100µL) showing 15.184981% viability. These selected phytochemical doses were further combined to study the synergistic effect of these phytochemicals.

The phytochemical combinations used for synergistic studies were

T_1-CGA,
T_2-CGA+EA,
T_3-EA+GA,
T_4-EA+CYCLO,
T_5-CGA+GA,
T_6-CGA+CYCLO,
T_7-CGA+GA+EA,
T_8-CGA+GA+CYCLO,
T_9-CGA+EA+CYCLO,
T_{10}-GA+EA+CYCLO,
T_{11}-CGA+GA+EA+CYCLO

On studying the synergistic effects of these phytochemicals on MDA MB 231 cells, the most significant combinations chosen for further studies were T_1 with 44.91164921% viability, T_2 with 27.15968586% viability, T_7 with 53.11954625% viability, T_{11} with 31.14092496% viability and T_5 with 35.80388307% viability.

Thus, the cytotoxic studies suggest that the phytochemicals are highly cytotoxic and should be further proceeded for studying various aspects.

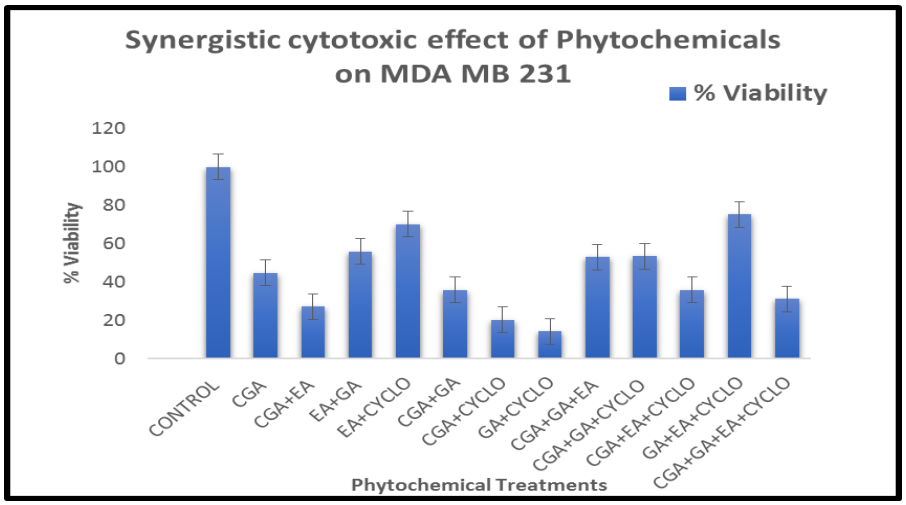

Fig 1: Synergistic cytotoxic effect of phytochemicals on MDA MB 231

3.2 Cell Migration Assay

Cell migration studies were performed using the selected phytochemical combinations against media control. T_1 (Chlorogenic acid) showed slight migration while other treatments viz., T_2 (CGA+EA), T_7 (CGA+GA+EA), T_{11} (CGA+GA+EA+CYCLO), T_5 (CGA+GA) did not migrate till 72 h. Also, decrease in the cell density with time was seen. Thus, it can be said that the treatments chosen have anti-metastatic activities.

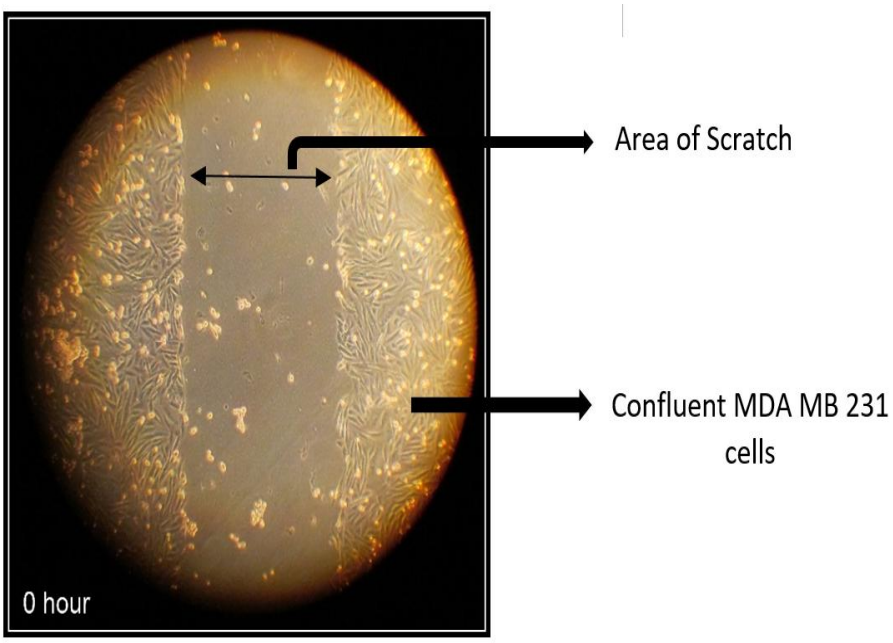

Fig 2: 0 h, Making of the scratch

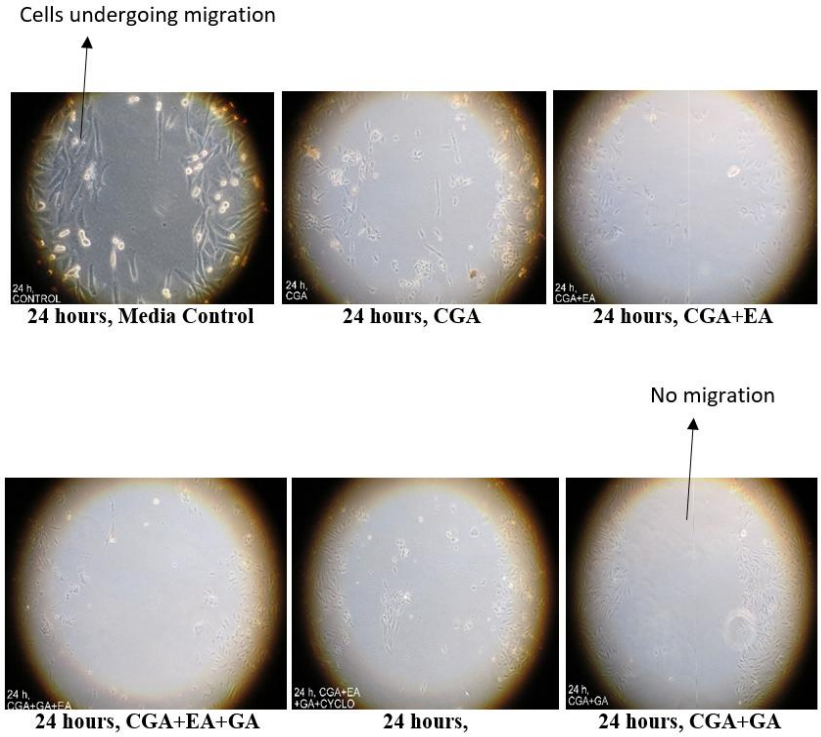

Fig 3: Effect of combinations of Chlorogenic acid, Gallic acid, Ellagic acid and Cyclophosphamide on migration of MDA MB 231 at 24 hours

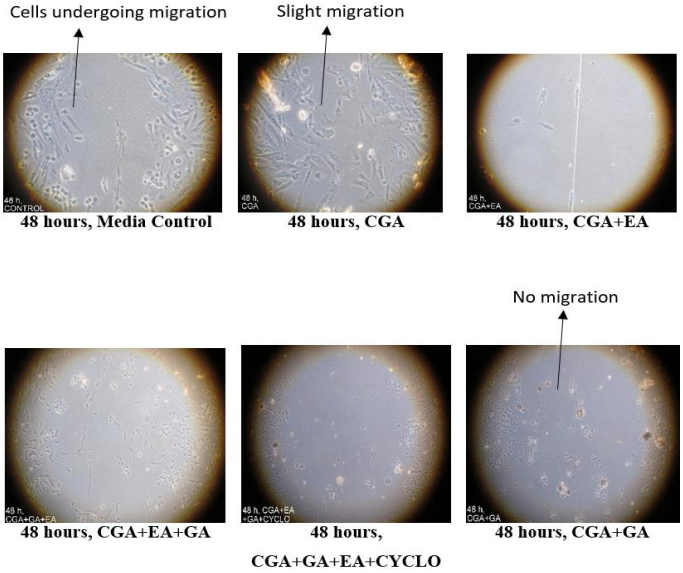

Fig 4: Effect of combinations of Chlorogenic acid, Gallic acid, Ellagic acid and Cyclophosphamide on migration of MDA MB 231 at 48 hours

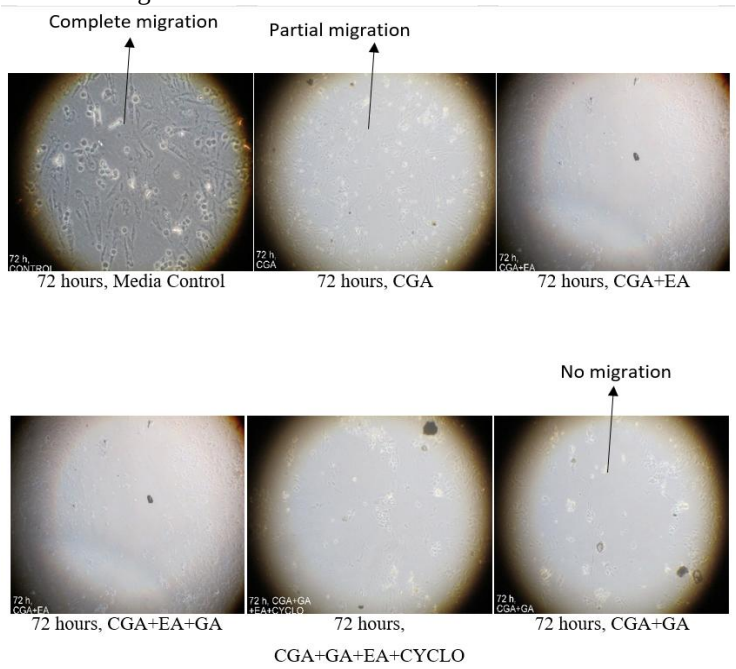

Fig 5: Effect of combination of Chlorogenic acid, Gallic acid, Ellagic acid and Cyclophosphamide on migration of MDA MB 231 at 72 hours

IV. Conclusion

The present study investigated the antiproliferative effect of Chlorogenic acid, Gallic acid and Ellagic acid on MDA MB 231 breast cancer cell line against a standard drug Cyclophosphamide using MTT assay. Chlorogenic acid, Gallic acid and Ellagic acid showed dose dependent inhibition and thus, the cytotoxic doses selected were Chlorogenic acid of 150 µM/100µL, Gallic acid of 5 µg/mL, Ellagic acid of 100 µM and Cyclophosphamide of 5 µg/100µL. All these concentrations were further used for studying the synergistic effects of the selected phytochemicals on MDA MB 231. On checking the synergistic cytotoxic effect of these phytochemicals on MDA MB 231 cell line, it was found that the combined phytochemicals are far more cytotoxic and thus, the most appropriate combinations chosen for further research work were T_1, T_2, T_7, T_{11}, and T_5. These combinations were further used for checking the migration of cells against media control. Scratch well Assay concludes that in the chosen treatments, only T_1 (CGA) shows slight migration while other treatments do not show any migration, while on the other hand, media control proliferates to cover the well completely in 72 h. Thus, the treatments, T_2, T_7, T_{11}, and T_5 must be anti-metastatic and thus, are very beneficial.

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