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ANTIPROLIFERATIVE ACTIVITY OF QUERCETIN AND PRIMA-1MET AGAINST HCT-116 AND HCT-15 COLON CANCER CELLS

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ABSTRACT

Quercetin is a plant flavonoid with significant chemopreventive and anticancer activity against many cancer types. The aim of the present study is to evaluate the anticancer properties of quercetin in synergism with the selected p53 activator PRIMA-1MET in human colon cancer cells with different p53 status. Colon cancer cells HCT-116 (wild type p53), HCT-15(mutant p53) were treated with different concentrations of both drugs alone and in combination and the effect was evaluated in cell viability and apoptosis assays. Cell viability - MTT assay was conducted with different doses of quercetin (10-300 µM) and PRIMA-1MET (5-75 µM) for 24 and 48 hours. Significant dose and time dependent reduction in cell viability was observed. High antiproliferative effect was observed at 48 hours treatment. Quercetin and PRIMA-1MET combination effect was checked in both cell viability and apoptosis assay by DAPI staining method. The highest antiproliferative effect was 64.8 at 50+10 µM (Que+PRI) concentration in HCT-116 and 80.8 at 50+50 µM in HCT-15 cells. Combination effect was evaluated by CompuSyn software, synergistic effect was observed at 50+10 µM (Que+PRI) concentration in both the cancer cells. Similar combination effect was observed in apoptosis assay, significant increase in apoptotic cells was observed in (Que+PRI) combination treatment than individual. 50+10 µM concentration of quercetin and PRIMA-1MET is an effective synergistic combination in both the selected cancer cells.Clear synergistic anticancer activity was observed with the selected drug combinations in both colon cancer cells (mutant and wild type p53) in all the assays due to the targeting of multiple activators and effectors which regulates cell proliferation and apoptosis.

KEYWORDS: *Quercetin, PRIMA-1MET, colon cancer, apoptosis, p53*

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INTRODUCTION

World-wide colorectal cancer (CRC) is the 3rd most regular type of cancer (12, 30000. 9.7%) [1].The incidence of colorectal cancer has rapidly increased in developed Asian countries. CRC rate is progressively increasing trend in India. Dietary and/or environmental factors are a cause for increase colorectal cancer rate due to the imitation of western food style and taking low vegetarian fiber food. Developed cities recorded more colorectal cancer incidents and high incidences were seen in non-vegetarians[2-3].At least 50% of human tumorsharbor mutations in p53. p53 mutations occur in about 60% of the lung carcinomas and about 50% of colon carcinomas. Mutation in p53 leads to decrease the rate of apoptosis, because of down-regulation pro-apoptotic Bcl-2 proteins (Bak, Bax, Bad& Bid) and up-regulation anti-apoptotic Bcl-2 proteins (Bcl-2, Bcl-xL). Use of p53 activators is increased in cancer treatment; p53 activators are the molecules activating p53 via direct interaction with p53 itself and covalently modifies the core domain of mutated forms of cellular tumor antigen p53 through the alkylation of thiol groups. These modifications reinstate both the wild-type conformation as well as function to mutant p53 and reconstitutes endogenous p53 activity leading to cell cycle arrest and apoptosis in tumor cells [4]. Selected p53 activator has shown synergism with other anticancer agents [5]. Many of the currently used chemotherapeutic agents results in clinical toxicity and/or undesirable side effects. Flavonoids attract a great deal of interest due to their extensive biological properties such as antioxidant, anti-proliferative, anti-inflammatory or antibiotic activity, which may contribute to their chemopreventive and adoptive response against development of cancer and cardio-vascular disease.In this assay, natural compound quercetin and p53 activator (PRIMA-1MET) are selected for testing their synergistic anticancer effects. Anticancer activity of natural compound in combination with selected p53 activator in cancer treatment has not been tested so far. Tumors hauling mutant p53 are more resistant to usual cancer therapies. National cancer institute identified that the PRIMA-1MET is a novel drug against tumors with mutant p53[6]. When we compare the sensitivity of PRIMA-1MET with known anticancer drugs, only paclitaxel has some specificity towards the cells expressing mutant p53. Paclitaxel is not effective towards colon cancer cells with mutant p53 while PRIMA-1MET is more effective[78]. The objective of present research plan is to determine the synergistic anticancer activity of novel combination of natural compound quercetin with the selected p53 activator. Quercetin-induced cell death in many human cancer cells by apoptosis [9] and plant flavonoids aremore effective in colon cancer treatment than other cancers because of their direct interaction[10] In this synergism, quercetin and selected p53 activator induces colon cancer cell death by upregulation of proapoptotic and downregulation of antiapoptotic Bcl-2 family proteins and increases p53, cytochrome C and caspases activity in apoptosis. p53 activator restores tumor suppressor function to mutant p53 proteins in colon cancer cells where the quercetin alone is not effective.

MATERIALS AND METHODS

Preparation of drugs - stock solution concentrations

Quercetin (6151-25-3) was procured from Sigma and PRIMA-1MET (5291-32-7) from Santa cruz biotechnology, Dallas, U.S.A (Genetix Biotech Asia Pvt Ltd, New Delhi, India). Quercetin and PRIMA-1MET stock solution (1milli molar) was prepared in solvent DMSO and stored at -20^{0}C until use.

MTT reagent preparation

3-(4,5-Dimethythiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT-Sigma) powder was dissolved in Dulbecco's modified eagle medium (DMEM-Sigma) to make up a stock of 5mg/ml. It was then filter sterilized with 0.22µm syringe filter and stored in the dark at 4ºC until use. Every time MTT reagent was prepared freshly and used on the same day.

Cell lines and culture media

Colon cancer cell lines; HCT-116 and HCT-15 were procured from ATCC (American Type Culture Collection), Manassas, VA.,andcultured in DME medium (Sigma) supplemented with 10% fetal bovine serum, 100 IU/ml penicillin, 100 µg/ml streptomycin and 0.25µg/ml amphotericin B (Himedia) in a CO_2 incubator at 37^{o}C and 5% CO_2[11].

Cell Viability (MTT)assay

Cells were seeded in 96-well plate at a density of ten thousand cells per well in 100µl of DME culture medium and incubated at 37ºC with 5% CO_2 for 24h, cells were treated either with quercetinor PRIMA-1MET and also with their combinations, at different concentrations for 24 and 48h and incubated at 37ºC with 5% CO_2. Each drug concentration/combination was tested in triplicates. After treatment duration drug containing media was removed and 30µl of MTT (5mg/ml in DMEM) reagent was added to each well and the plates were incubated for 3h at 37ºC. The MTT containing media was removed and DMSO (100µl) was added to each well. After ten minutes of mechanical shaking, the optical density (OD) was measured for each well at 570nm in a plate reader (synergy 4 multimode microplate reader-BioTEK). Experiment was repeated at least three times. The percent cell viability was calculated using the following equation[12].

$$\% \text{ Cell Viability} = \frac{\text{OD Test}}{\text{OD Control}} \times 100$$

Analysis of combination effect using compuSyn software

In combination studies cells were treated with different concentrations of quercetin + PRIMA-1MET at the same time for 48h. The applied drug combinations were 50+10, 50+25, 100+10 and 100+25 µM (que+PRI)in HCT-116, 50+10, 50+25, 50+50, 100+10, 100+25, 100+50, 150+10, 150+25 and 150+50 in HCT-15 cells.

Cell viability or inhibition of cell proliferation was calculated as described using MTT assay. Data were further analyzed using the median effect analysis method, where the extent of combination effect (synergistic, and/or additive) is determined by the combination index (CI) value, and calculated using CompuSyn software[13]. A synergistic interaction is determined by a CI<1, an additive interaction is determined by a CI=1 and antagonism is determined by a CI >1[14].

Combination effect on apoptosis - DAPI staining

Cells (1×10^5in 1.0ml of DME culture medium) were plated in 6well tissue culture plates and after overnight incubation at 37°C and 5% CO_2, they were treated with quercetin (50 µM) or with PRIMA-1MET (10 µM) alone, and in combination for 48h. After treatment, cells were harvested by trypsinisation. Cells were pelleted and the pellet was washed three times in PBS (1100rpm for 5min). The cells were fixed in 3.7% formaldehyde in PBS for 10min at room temperature. The fixative was removed and the cells were washed 3x, 5min each in PBS and incubated with 0.1% Triton X-100 (Sigma) for 5min, to make the cells permeabilize. The Triton X-100 was aspirated and the cells were washed 3x, 5min each in PBS and incubated with DAPI (Sigma) staining solution (stock =1mg/ml in dH_2O - diluted 1:1000 in PBS before use) for 10min at room temperature in the dark. At last, the DAPI solution was aspirated and the cells were washed 3x, 5min each in PBS, then a drop of cell suspension was placed on a slide and mounted with cover slip and examined under fluorescent microscope with blue filter. The slides were screened for the quantification of cells showing features of apoptosis, and the results were expressed as % apoptotic cells [15].

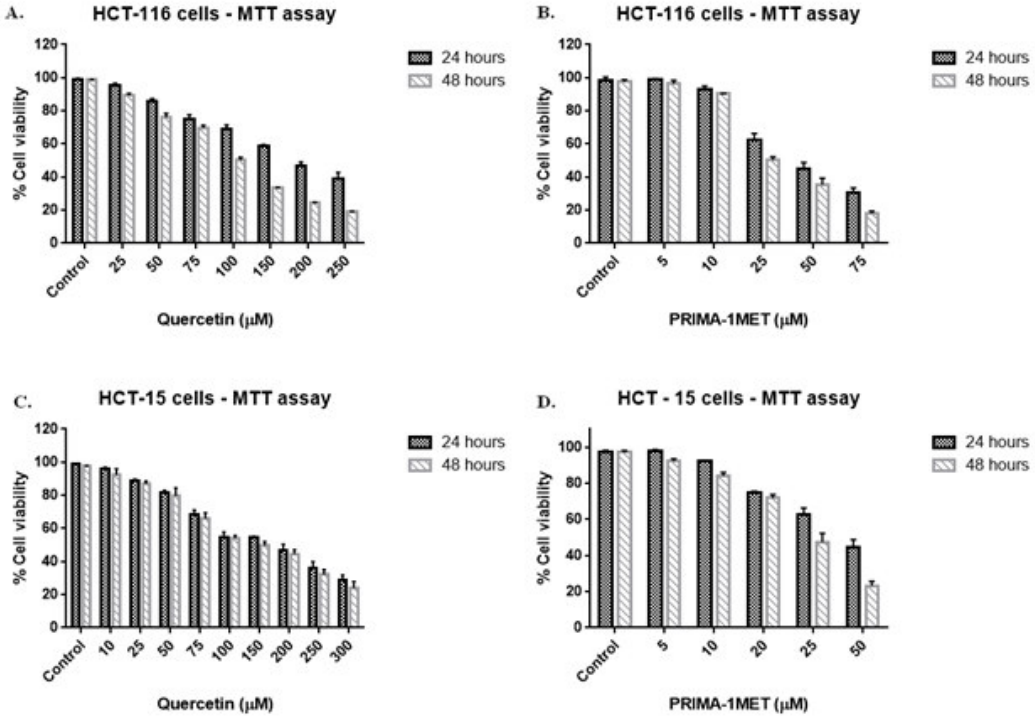
STATISTICAL ANALYSIS

All the experiments were done in triplicate. Results were expressed as mean ± standard deviation (SD), one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test were performed using GraphPad Prism Software version 5.0 (San Diego, USA) with level of significance at *P* 0.05.

RESULTS

Quercetin and PRIMA-1MET inhibits cell proliferation

Cells were treated with different doses of quercetin (10-300 µM) and PRIMA-1MET (5-75 µM)for 24 & 48 hours and viability was determined by MTT cell proliferation assay.Both the drugs were tested individually, significant reduction in cell viability compared to control was observed in dose and time dependent mode, Figure-1. The calculated IC50 values in HCT-116 cells were at 91.0 µM and 27.0 µM for quercetin and PRIMA-1MET respectively; where as in HCT-15 cells the IC50 vales for quercetin and PRIMA-1MET were at 142.4 µM and 47.3 µM respectively. In combination assaycells were treated with different concentrations quercetin+PRIMA-1MET for 48 hours treatment duration adding both drugs at once. Cell viability was determined by MTT assay. The highest anti-proliferative effect was 64.8 at 50+10 µM (Que+PRI) concentration in HCT-116 and 80.8 at 50+50 µM in HCT-15 cells. Based on the MTT assay results drug combination effect was calculated using CompuSyn software and the results were represented in CI-Fa plot,Figure-2. The observed CI values were detailed in Table-1.

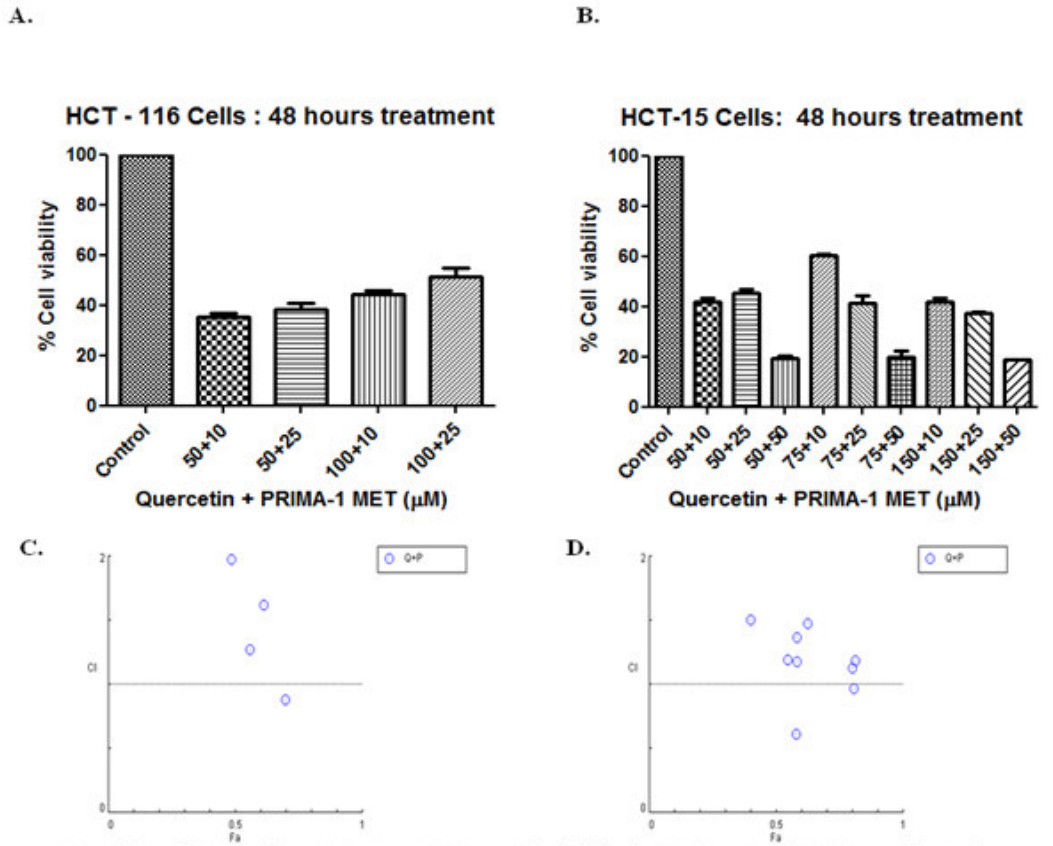


Effect of quercetin and PRIMA-1MET on cell viability in HCT-116 (A,B) and HCT-15 (C,D) colon cancer cells. Cells were treated for 24 & 48h and % cell viability was determined by MTT assay. Data were expressed as mean ± SD (n=3).

Figure 1
MTT cell viability assay

Table 1
CI values calculated using CompuSyn software

Cell line	Quercetin+PRIMA-1MET (μM)	CI Values
HCT-116	50+10	0.88*
	50+25	1.62
	100+10	1.27
	100+25	1.97
HCT-15	50+10	0.61*
	50+25	1.19
	50+50	0.97#
	100+10	1.50
	100+25	1.37
	100+50	1.12
	150+10	1.18
	150+25	1.47
	150+50	1.19



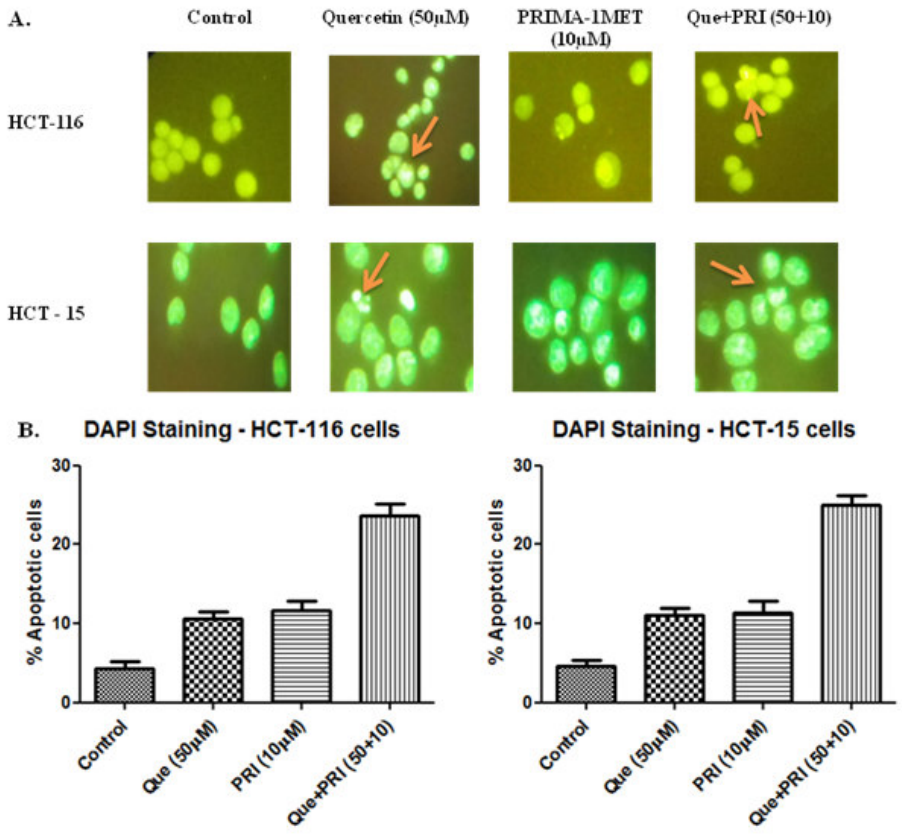
A. B. Effect of Quercetin + PRIMA-1MET on cell viability in HCT-116 and HCT-15 cells. Cells were treated for 48 hours and viability was determined by MTT assay. Data were expressed as mean ± SD (n=3) $^{*}p \leq 0.001$. C.D. CI-Fa plot : Analysis of combination effect on cell viability in HCT-116 (c.) and COLO-320 cells (D.) using CompuSyn software.

Synergistic combination effect (CI<1*) was observed at 50+10 μM (que+PRI) concentration in both HCT-116 and HCT-15 cells and additive effect (CI =1$^{\#}$) at 50+50 μM in HCT-15 cells.Whereas antagonistic effect was observed (CI >1) at remaining applied concentrations.

Figure-2
Combination effect of Quercetin+PRIMA-1MET and CI-Fa plot

Combination effect on apoptosis - DAPI staining

After treatment and DAPI fluorescent dye staining process, apoptosis induction was evaluated by observing slides under fluorescent microscope to identify the fragmentation and condensation nuclei/nucleic DNA of apoptotic cells. The observed apoptotic cells with morphological changes of nuclei after being treated with both the drugs individually and in combination are shown in Figure-3.Apoptosis or programmed cells death performs an vital role for an effective cancer therapies and becomes the pharmaco-dynamic endpoint for anticancer treatment[16]. Significant increase in % apoptotic cells were observed in drug combination (que+PRI) at 50+10 μM concentration when compare with negative control and individual drug treatment concentrations (que 50 and PRI 10 μM) in both HCT-116 and HCT-15 cells.



Que: Quercetin, PRI: PRIMA-1MET, Effect of que, PRI individually and in combination on % Apoptosis in HCT-116 and HCT-15 cells in DAPI staining method. Data were expressed as mean ± SD (n=3) *p≤0.001. A: Apoptotic cells with nuclear fragmentation and membrane shrinkage, B: % Apoptotic cells Vs treatment concentrations.

Figure 3
Combination effect on apoptosis - DAPI staining

DISCUSSION

p53 based cancer therapies got more importance because of at least 50% of human tumorsharbor mutations in p53, *TP53* is of the very commonly mutated gene in human malignancies. p53, tumor suppressor single protein is the best known barrier against malignant transformation[17].p53 mutant cells were more resistant to many of chemotherapy drugs and also chemotherapeutic agents often lead to undesirable side effects[18].The potential anticancer effects of herbal combinations are more effective than individual [19]. The combination of two or more agents can reduce the toxicity or other side effects single pure compound [20].Colon cancer cells with mutant (HCT-116) and wild type p53 cells (HCT-15) were selected to evaluate the synergistic anticancer activity of plant flavonoidquercetin and effective p53 activator PRIMA-1MET.Anticancer properties of both the drugs were demonstrated individually via different mechanisms and extensive research is required for their effect in combination assays[21]. Quercetin is a natural plant flavonoid widely distributed in many of plant food and is effective in selected cancer cells HCT-116, HCT-15 even at lower concentrations.Reduction in cell proliferation and increase in % apoptotic cells were observed in both drug treatments individually and in combination. In this study, we observed that combination treatment of que+PRI is more effective than individual treatment due to strong synergism. More significant anticancereffect was observed in HCT-15 (mutant p53) cells than HCT-116 (wild type p53). The present results revealed that both selected drugs were effective individually and in combination in both HCT-116 and HCT-15 colon cancer cells. Combination treatment is more effective than individual treatment and HCT-15 cells were more sensitive than HCT-116.

CONCLUSION

In conclusion the present study results clearly showed that synergistic anticancer activity of quercetin+PRIMA-1MET in both HCT-116 and HCT-15 colon cancer cells. HCT-15 mutant type p53 cells are more sensitive than wild type HCT-116 cells. The selected drug combination is more effective to mutant p53 than wild type.The present study information is useful and provide basis for furtheranticancer assays against different cancer types.

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CONFLICT OF INTEREST

Conflict of interest declared none.

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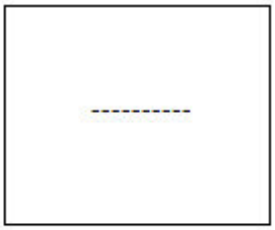

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