

# Fisetin synergizes with gemcitabine and inhibits viability of MIA PaCa-2 pancreatic cancer cells

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## ABSTRACT

In recent years multiple therapy cycles with GCB have shown a lot of resistance towards pancreatic cancer treatment. Thus different methods have been attempted to improve its potency. This study aims to improve the potency of GCB using dietary molecule Fisetin, a flavonoid molecule commonly found in fruits and vegetables. Pancreatic cancer cell line MIA PaCa-2 was treated with different doses of Fisetin and Gemcitabine individually and in combination. Cellular viability was estimated spectrophotometrically using MTT assay. Combination treatment of Fisetin and Gemcitabine showed synergistic effect in inhibiting the growth of MIA PaCa-2 cell line. We conclude that dietary molecule Fisetin synergizes with Gemcitabine to inhibit MIA PaCa-2 proliferation.

**KEYWORDS:** Synergism, Pancreatic cancer, MIA PaCa-2, Fisetin, Gemcitabine

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## INTRODUCTION

Resistance towards Gemcitabine by pancreatic cancers due to different mechanisms after multiple rounds of chemotherapy has emerged as a major problem. (Binenbaum, Na'ara et al. 2015) (Chand, O'Hayer et al. 2016) Use of nutraceuticals (dietary molecules with health benefits) in combination with GCB has emerged as a promising strategy for improving efficacy of GCB treatment. (Pandita, Kumar et al. 2014) Nutraceuticals are common flavonoids found in different parts of foods and vegetables. Since they are dietary in nature they barely affect normal human cells. Fisetin (3,3',4',7'-tetrahydroxyflavone) is one such molecule, commonly found in apples cucumbers, onions and others, whose potential as anticancer agent is tested against many cancer types (Liao, Shih et al. 2009; Chen, Hsieh et al. 2015) Fisetin has been shown to inhibit angiogenesis (both *in vivo* and *in vitro*), migratory and invasiveness of cancer cells. (Bhat, Nambiar et al. 2012) Till date only limited attempts have been made where the potential of Fisetin in combination treatments is tested. (Klimaszewska-Wisniewska, Halas-Wisniewska et al. 2016; Pal, Diamond et al. 2016) In this study the effect of Fisetin alone and in combination with GCB is explored on pancreatic cancer cell line MIA PaCa-2.

## MATERIAL AND METHODS

### Cell Lines, maintenance and reagents

MIA PaCa-2 (Human pancreatic cancer) and FR2 (SV40 transformed breast epithelial non-cancerous) cell lines were maintained as described elsewhere (Pandita, Kumar et al. 2014). Fisetin, Gemcitabine, MTT (dimethylthiazole-2-yl-2, 5-diphenyl-tetrazolium-bromide) and other chemicals were purchased from Sigma-Aldrich USA.

### Treatment method and MTT assay

MIA PaCa-2 and FR2 cells were seeded ( $4 \times 10^4$  cells/well) in 24-well flat bottom plates (Nunc) and kept for 24 hrs. Next day cells were exposed to different concentrations of Fisetin and GCB, alone and in combinations. For combination treatments cells sensitized with Fisetin for 24 hours were treated with gemcitabine for further 24 hours in the presence of Fisetin. Thus total treatment duration for Fisetin and Gemcitabine was 48 hours and 24 hours respectively. MTT assay was performed as described elsewhere (Kumar, Iqbal et al. 2015). Cellular viability values were expressed as OD change at 570 nm and as % viability.

### Analysis of combined drug effects

Combination-index (CI) and Isobologram methods as described by Chou and Talalay (1984), was used for analysis of combination treatment using the CompuSyn software (Chou and Talalay 1984). Non-constant ratio of drug and dietary molecule was used for combination treatment. Fractions affected (Fa) were calculated from the viability data obtained by MTT assay. These Fa values from alone and drug concentrations were used for making isobologram and Fa-CI plot.

### Statistical Analysis

Each experiment was replicated at least three times and the data expressed as mean  $\pm$  standard error. P values were calculated using one way ANOVA where  $P < 0.05$  was considered statistically significant and \* =  $P < 0.05$ , \*\* =  $P < 0.01$ , \*\*\* =  $P < 0.001$ .

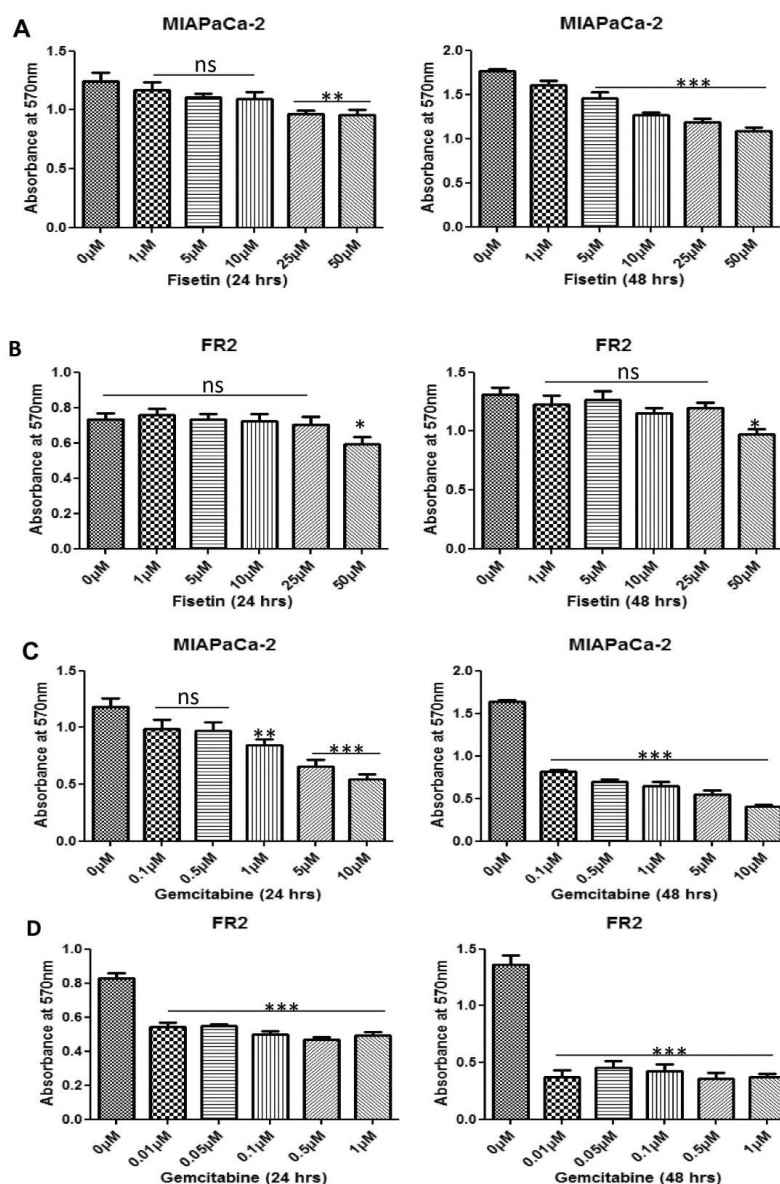
## RESULTS

### Effect of Fisetin and Gemcitabine on growth on MIA PaCa-2 and FR2

Fisetin treatment for 24 hour and 48 hours showed significant growth inhibitory effects on MIA PaCa-2 cells at the different doses (Figure 1A). However little or no effect was observed on growth of

noncancerous cell line FR2 except at 50  $\mu\text{M}$  dose for both 24 hours and 48 hours (Figure 1B). While Gemcitabine treatment on MIA PaCa-2 showed very strong growth inhibitory effects at both 24 hours and 48 hours in micro-molar doses but nano-molar (100nM) concentrations showed inhibition only at 48 hour (Figure 1C).

Noncancerous FR2 cells were found to be highly sensitive to as low as 10nM concentration of Gemcitabine at both 24 and 48 hours (Figure 1D). Since Fisetin selectively affected growth of only MIA PaCa-2 cells, its use in combination with GCB to potentiate GCB's effect was thought pertinent.



**Figure 1.** Effect of Fisetin and Gemcitabine on growth of MIA PaCa-2 and FR2 cell line. (A) Viability of MIA PaCa-2 cells upon treatment with Fisetin for 24hrs and 48hrs. (B) Viability of FR2 cells upon treatment with Fisetin for 24hrs and 48hrs. (C) Viability of MIA PaCa-2 cells upon treatment with Gemcitabine for 24hrs and 48hrs. (D) Viability of MIA PaCa-2 cells upon treatment with Fisetin for 24hrs and 48hrs. Cells viability was estimated using MTT assay as mentioned in material and method. P values were calculated using one way ANOVA where  $P < 0.05$  was considered statistically significant and \* =  $P < 0.05$ , \*\* =  $P < 0.01$ , \*\*\* =  $P < 0.001$ .

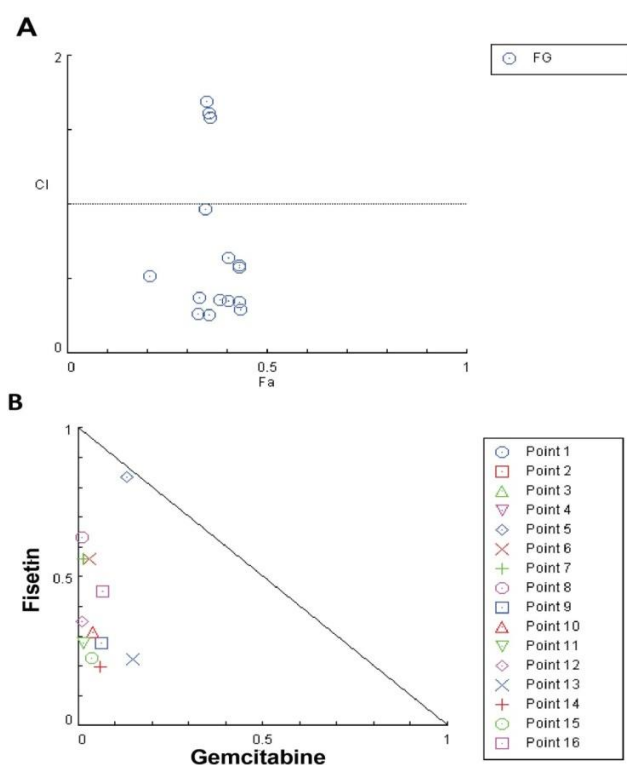
### Combination studies of Fisetin and Gemcitabine on MIA PaCa-2 cell line

Since 10nM dose of gemcitabine shows around 35% inhibition of noncancerous cells for 24 hours treatment, doses from 3.12-12.5 nM were preferred for combination treatment. MIA PaCa-2 cells sensitized with Fisetin for 24 hours were treated with 3.12nM, 6.25nM and 12.5nM dose for further 24 hours thereby making Fisetin treatment duration 48 hours and Gemcitabine only 24 hours. Isobolograms and CI values were calculated for the drug combinations (non-constant ratio) using

CompuSyn software (Table 1). Interestingly higher dose of Fisetin (50  $\mu$ M) in combination with Gemcitabine showed antagonism (- - -) depicted by the CI values lying in range 1.45 - 3.30, whereas lower doses showed synergism (+++) depicted by CI values between 0.3-0.7 in the FA-CI plot (Figure 2A). The normalized isobologram graph showed that for most of our drug combination treatment lies within synergism areas of the graph (Figure 2B). These observations emphasize importance of the amount of nutraceutical concentrations used in combination treatments leading to different outcomes.

Table 1. Combination Index (CI) values of different combinations treatment.

Dose Fis ( $\mu$ M)	Dose Gem (nM)	Effect	CI value
50.0	25.0	0.26499	1.82204
50.0	12.5	0.35052	1.69298
50.0	6.25	0.35628	1.61504
50.0	3.12	0.35856	1.58237
25.0	25.0	0.34574	0.96670
25.0	12.5	0.43192	0.59127
25.0	6.25	0.43177	0.57550
25.0	3.12	0.40407	0.64402
12.5	25.0	0.43240	0.34333
12.5	12.5	0.40591	0.35429
12.5	6.25	0.43428	0.29239
12.5	3.12	0.38380	0.35993
6.25	25.0	0.33258	0.37177
6.25	12.5	0.35537	0.26013
6.25	6.25	0.32775	0.26673
6.25	3.12	0.20687	0.52010



**Figure 2.** Fa-CI plot and isobologram for combination treatments of Fisetin and Gemcitabine (FG). (A) Fa-CI plot for combination treatment (B) Isobologram for non-constant ratios of Fisetin and Gemcitabine used as mentioned in table 1. All points below the lines represent synergistic doses while those above the lines represent antagonistic doses.

## DISCUSSION

Pancreatic cancer is 4<sup>th</sup> leading cause of deaths due to cancer worldwide. (Hariharan, Saied et al. 2008) Since there are no methods for early detection of pancreatic cancers, patients are usually diagnosed during advanced stages leading to high rate of mortality. (Kim and Ahuja 2015) To make the matters worst pancreatic cancers show resistance towards the primary drug Gemcitabine used for treating pancreatic cancers. (Yoneyama, Takizawa-Hashimoto et al. 2015) Drug combinations formulations using nutraceuticals (dietary molecules) has arose as method of choice to improve efficacy of chemotherapeutic drugs in killing cancer cells while maintaining minimal

toxicity towards normal cells. In this study we attempt to understand the effect of Fisetin in combination with GCB against pancreatic cancer cell line MIA PaCa-2. Interestingly Fisetin inhibited growth of MIA PaCa-2 in a dose and time dependent manner but showed little or no effect on FR2 cell line, suggesting that Fisetin selectively affects cancerous cells and show little or no effect on normal cells. Previous studies have also proved that nutraceuticals hardly affect viability of normal cells. (Chen, Schell et al. 1998) FR2 cells on the other hand were very sensitive towards GCB even in nanomolar ranges at 48 hours, while inhibition at 24 hours was relatively less. Therefore nanomolar concentrations of GCB were chosen for combination treatment to minimize the death of non-cancerous cells and the treatment duration was kept 24 hours only. The IC<sub>50</sub> for Fisetin was found to be 60.45  $\mu$ M at 48hrs while IC<sub>50</sub> of Gemcitabine was found to be 664.62 nM at 48hrs. Combination treatment of Fisetin and GCB showed strong synergism at many non-constant ratios tried. The synergistic effect was more profound at lower dose combinations, while higher dose concentrations showed antagonism (Fig. 2). Earlier studies have shown that gemcitabine in combination with 5-Fluorouracil also showed antagonistic effect on Pancreatic Carcinoma Cell Line Capan-2 (Bellone, Carbone et al. 2006). Thus our work demonstrates that care should be taken while choosing effective concentrations of nutraceuticals in combination therapies. We further suggest more in-depth studies at both in-vitro and in-vivo levels to further establish this combination therapy regime.

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## Conflict of interest statement

The author has declared that no competing or conflicts of interests exist. The funders had no role in study design, writing of the manuscript and decision to publish.

## Authors' contributions

BK conceived, designed, conducted and wrote the manuscript.

## REFERENCES

- Bellone, G., A. Carbone, et al. (2006). "Antagonistic interactions between gemcitabine and 5-fluorouracil in the human pancreatic carcinoma cell line Capan-2." *Cancer Biol Ther* 5(10): 1294-1303.
- Bhat, T. A., D. Nambiar, et al. (2012). "Fisetin inhibits various attributes of angiogenesis in vitro and in vivo--implications for angioprevention." *Carcinogenesis* 33(2): 385-393.
- Binenbaum, Y., S. Na'ara, et al. (2015). "Gemcitabine resistance in pancreatic ductal adenocarcinoma." *Drug Resist Updat* 23: 55-68.
- Chand, S., K. O'Hayer, et al. (2016). "The Landscape of Pancreatic Cancer Therapeutic Resistance Mechanisms." *Int J Biol Sci* 12(3): 273-282.
- Chen, C. M., Y. H. Hsieh, et al. (2015). "Fisetin suppresses ADAM9 expression and inhibits invasion of glioma cancer cells through increased phosphorylation of ERK1/2." *Tumour Biol* 36(5): 3407-3415.
- Chen, Z. P., J. B. Schell, et al. (1998). "Green tea epigallocatechin gallate shows a pronounced growth inhibitory effect on cancerous cells but not on their normal counterparts." *Cancer Lett* 129(2): 173-179.
- Chou, T. C. and P. Talalay (1984). "Quantitative analysis of dose-effect relationships: the combined effects of multiple drugs or enzyme inhibitors." *Adv Enzyme Regul* 22: 27-55.
- Hariharan, D., A. Saied, et al. (2008). "Analysis of mortality rates for pancreatic cancer across the world." *HPB (Oxford)* 10(1): 58-62.
- Kim, V. M. and N. Ahuja (2015). "Early detection of pancreatic cancer." *Chin J Cancer Res* 27(4): 321-331.
- Klimaszewska-Wisniewska, A., M. Halas-Wisniewska, et al. (2016). "Paclitaxel and the dietary flavonoid fisetin: a synergistic combination that induces mitotic catastrophe and autophagic cell death in A549 non-small cell lung cancer cells." *Cancer Cell Int* 16: 10.
- Kumar, B., M. A. Iqbal, et al. (2015). "Resveratrol inhibits TIGAR to promote ROS induced apoptosis and autophagy." *Biochimie* 118: 26-35.
- Liao, Y. C., Y. W. Shih, et al. (2009). "Involvement of the ERK signaling pathway in fisetin reduces invasion and migration in the human lung cancer cell line A549." *J Agric Food Chem* 57(19): 8933-8941.
- Pal, H. C., A. C. Diamond, et al. (2016). "Fisetin, a dietary flavonoid, augments the anti-invasive and anti-metastatic potential of sorafenib in melanoma." *Oncotarget* 7(2): 1227-1241.
- Pandita, A., B. Kumar, et al. (2014). "Synergistic combination of gemcitabine and dietary molecule induces apoptosis in pancreatic cancer cells and down regulates PKM2 expression." *PLoS One* 9(9): e107154.
- Yoneyama, H., A. Takizawa-Hashimoto, et al. (2015). "Acquired resistance to gemcitabine and cross-resistance in human pancreatic cancer clones." *Anticancer Drugs* 26(1): 90-100.