

ORIGINAL RESEARCH

Anticancer Efficacy of Fucoxanthin in Targeting Ovarian Cancer Cells

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ABSTRACT

Fucoxanthin, a natural carotenoid primarily found in brown seaweed, has garnered significant attention in recent years due to its potential anti-cancer properties. Research exploring its effects on various types of cancer, including ovarian cancer, has shown promising results in inhibiting tumor growth, inducing apoptosis, and suppressing the spread of cancer cells. Ovarian cancer is one of the most lethal gynecologic malignancies worldwide. Its high mortality rate is often attributed to late diagnosis, limited treatment options, and the development of drug resistance. Therefore, the quest for novel and effective therapeutic agents to combat ovarian cancer remains a crucial area of research. Fucoxanthin, with its unique biological properties, has emerged as a potential candidate in this pursuit. Fucoxanthin has been shown to induce apoptosis in ovarian cancer cells. This programmed cell death mechanism is crucial in eliminating damaged or abnormal cells. Fucoxanthin triggers apoptotic pathways within cancer cells, leading to their self-destruction, thereby impeding tumor growth and metastasis. Considering pieces of evidence of the anticancer effects of Fx on various cancers, we investigated the anticancer properties of this carotenoid on ovarian cancer cells (PA-1) compared to the standard anticancer drug tamoxifen (Tx).

Key words: Apoptosis, cancer, carotenoids, fucoxanthin, PA-1 cells, tamoxifen

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INTRODUCTION

Fucoxanthin (Fx) an orange colour pigment of edible brown algae prevents the formation of cancer cells caused by oxidative stress and obesity. [1] Fucoxanthin causes loss of mitochondrial membrane potential which further leads to apoptosis. Mitochondrial membrane permeabilization is an important step at the early stage of the apoptosis pathway via mitochondria. [2] Fucoxanthin and fucoxanthinol (Fxo) had been found with remarkable antiproliferative properties in Human T cell leukemia virus type 1- infected T cell lines and adult T cell leukemia in-vitro. [3] Human colon cancer cell lines Caco-2, HT-29, and DLD-1 treated with Fx resulted in a reduced number of viable cells. [4] Lutein (25–100 μM) was tested to examine how different vehicles (THF, DMSO, and FBS) affect the effectiveness of lutein in apoptotic activity of HeLa cancer cells. The viability of the cells decreased when treated with lutein delivered through THF, DMSO, and FBS. Further, a lower concentration of lutein (25 μM) delivered through THF, DMSO, and FBS affected cell

viability as 54.1, 58, and 73.4% respectively at 72 hrs post-incubation. Besides, lutein with a concentration below 25 μM did not induce cell necrosis. The study found that the choice of delivery vehicle affects the effectiveness of lutein in reducing HeLa cell viability. [5]

Fucoxanthin, a major marine carotenoid consists of an allenic bond and a 5,6-monoepoxide, is present in seaweeds such as "*Hijikia fusiformis*, *Undaria pinnatifida*, and *Sargassum fulvellum*." It possesses antitumor and anti-inflammatory activity by inducing apoptosis and reducing free radicals. [6]

Two breast cancer cell lines (MCF-7 and MDA-MB-231) were treated with a low dose (10 and 20 μM) of Fx and Fxo to evaluate their anticancer effects. Both Fx/Fxo (10 μM) showed antiproliferative activity in these cells by inducing apoptosis. Fucoxanthinol showed more rapid and efficient inhibitory action on cell growth as compare to Fx. Fucoxanthinol (20 μM) reduced the viability of MCF-7 cells by 58% at 48 hrs incubation whereas the viability of MDA-MB-231 was reduced to 13% and 47% with a dose of 10 and

20 μM respectively. Only MDA-MB-231 cells showed growth suppression with 10 μM Fxol at 48 hrs [7]. Liu et al (2009) documented the anti-proliferative effect of Fx against SK-Hep-1 (human hepatoma) cells and BNL CL2 (murine embryonic liver) cells. Fucoxanthin was significantly resistant to the growth of SK-Hep-1 cells at 24 hrs. Besides, a similar effect was noticed for Fx concentrations $>1 \mu\text{M}$ after 48 hrs. On another hand, Fx appeared as a facilitator for the growth of BNL CL2 cells until 24 hrs and showed a slight decrease in proliferation at 48 hrs. Results suggested that cell proliferation arrest and apoptosis induced by Fx were mediated by increased cell gap junction and intracellular Ca^{++} level. [8] Prostatic cancer cells (DU145) were treated with Fx to elucidate the precise mechanism of its anticancer activity. DNA microarray system was used to assess the effect of Fx on gene expression and any change in the gene expression was confirmed by northern blot and/or quantitative RT-PCR. The impact of Fx on cell cycle movement was investigated utilizing flow cytometry and RNA interference experiments were performed for the GADD45 gene. Fucoxanthin arrested the cell cycle at the G1 phase and suppressed the growth of cancer cells in a time and dose-dependent manner. The cell cycle arrest by Fx was found to be linked with the induction of GADD45A. Though, Fx did not induce apoptosis but acted as a cytostatic agent in these cells. [9] The growth of human T24 bladder cancer cells was seized by Fx in a dose and time-dependent manner through cell cycle arrest in the G0/G1 phase, involving up-regulation of p21, a cyclin-dependent kinase (CDK) inhibitory protein, and the down-regulation of CDK-2, CDK-4, cyclin D1, and cyclin E. [10] Fucoxanthin induced O^- (ROS) generation that ended up in apoptosis of HL-60 (leukemic cell) lines. Overproduction of H_2O_2 and O_2^- along with sub G1 DNA content was observed in the cells treated with Fx (indicating cell cycle arrest in the G1 stage). On exposure to commercial antioxidants (NAC), a reduced number of apoptotic bodies and DNA fragmentation of cells were noticed. Thus, the study concluded that the cytotoxicity of Fx was mediated by ROS generation that triggered apoptosis in HL-60 cells. [11]

An in-vitro study on GOTO cell lines (human neuroblastoma) showed a 68% suppression induced by Fx (10 $\mu\text{g}/\text{ml}$) after 3 days. Reduced expression of N-myc gene after 4 hrs application of Fx (10 $\mu\text{g}/\text{ml}$) was observed. Cell cycle arrest in the G0-G1 phase was related to the cell growth suppression. It was suggested that the Fx protected the neuronal cells from further damage by reducing free radical activity. [12] Another study showed inhibition of Akt phosphorylation by Fx in a concentration-dependent manner. Caspase-dependent cell death was induced by Fx in HeLa cell lines. Fucoxanthin lowered the extent of NF- κB activation with an insignificant effect on AP-1 activation. The author concluded that Fx expressed antitumor activity in HeLa cell lines. [13] Considering pieces of evidence of the anticancer effects of Fx on various cancers, we investigated the anticancer properties of this carotenoid on ovarian cancer cells (PA-1) compared to the standard anticancer drug tamoxifen (Tx).

RESULTS

MTT assay

MTT assay was used to measure cell proliferation. Effects of various agents were evaluated on PA-1 cell lines at 24 and 48hrs

Effects of Fx on PA - 1 cells

For Fx treatment, there was no statistically significant interaction between the factors of time points and treatment levels on the effect of percent viability ($F_{(9, 40)} = 1.39$, $p = 0.23$, partial $\eta^2 = 0.24$) suggesting that the overall difference between the mean percent viabilities of PA -1 cells after 24 hr incubation was comparable to that of 48 hrs incubation.

At both 24 hrs and 48 hrs incubations, the mean percent viabilities of all drug concentrations were comparable to that of respective controls (24 hr: $F_{(9, 20)} = 1.38$, $p = 0.26$; 48 hr: $F_{(9, 20)} = 0.93$, $p = 0.52$) suggesting no evident anticancer effects due to Fx on PA-1 cells with the concentrations and incubation times used in our experiment. (fig.1)

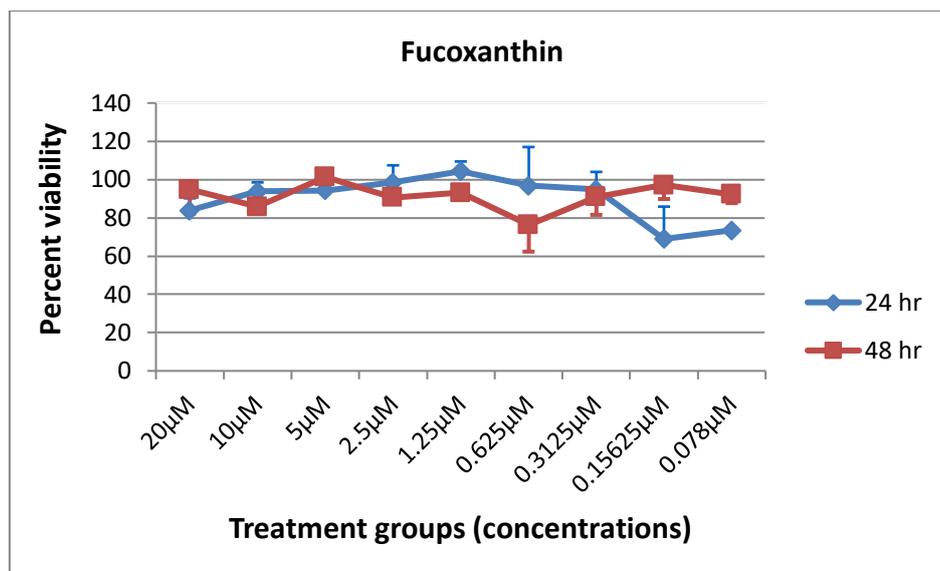


Fig. 1: Effect of Fx on PA-1 cells at 24 and 48 hrs

Effects of Tx on PA - 1 cells

For Tx treatment, there was an overall statistically significant interaction between the factors of time points and treatment levels on the effect of percent viability of PA – 1 cells ($F_{(9, 40)} = 3.157, p = 0.006$, partial $\eta^2 = 0.415$). Specifically, the mean percent viabilities of cells at 24 hrs were significant lesser than at 48 hrs with 10 µM ($p < 0.001$), 2.5 µM ($= 0.023$), 1.25 µM ($p = 0.008$) and 0.3125 µM ($p = 0.023$) respectively, suggesting that 24 hrs incubation was better to reduce the growth of PA-1 cells. After

24 hrs incubation, we found a significant effect of different concentrations on percent viability ($F_{(9, 20)} = 5.74, p < 0.001$).

Post-hoc test showed a markedly less percent viability than control in 10 µM ($p = 0.017$) concentration. However, 48 hrs incubation did not show any significant effect of different concentrations on percent viability ($F_{(9, 20)} = 1.2, p = 0.35$) suggesting 48 hrs incubation was not effective in controlling the growth of PA-1 cells in our experiment. (fig. 2)

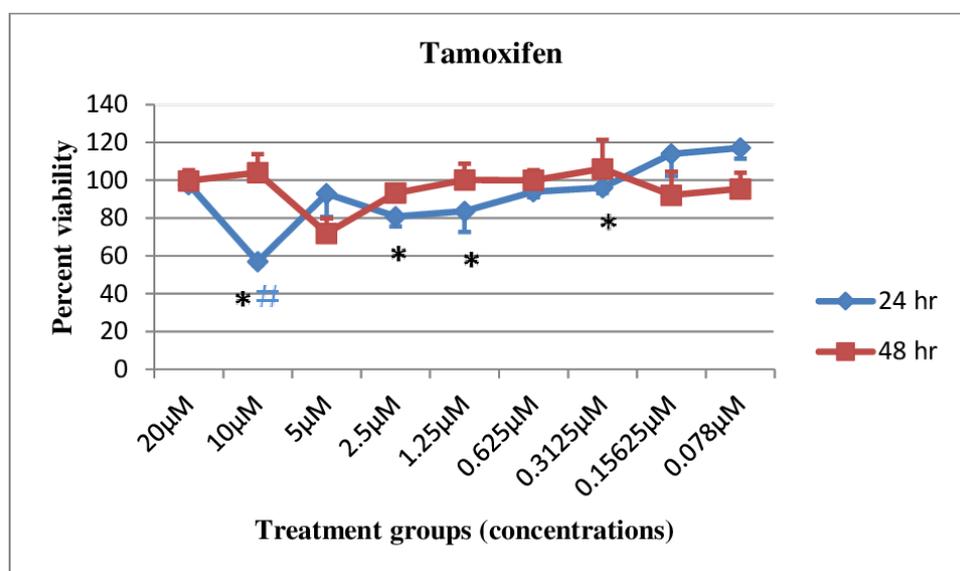


Fig.2: Effect of Tx on PA-1 cells at 24 and 48 hrs

significant difference: *b/w 24 and 48 h; # b/w 24 h and control # b/w 48 h and control

Comparative effects of Fx and Tx on PA - 1 cells at 24 hrs

After 24 hr of incubation, there was a statistically significant interaction between the factors of drug types and treatment levels on the effect of percent

viability ($F_{(9, 40)} = 4.570, p < 0.001$, partial $\eta^2 = 0.507$). The mean percent viability of PA – 1 cells with Fx was markedly less than Tx at 0.15625 µM and 0.078 µM (both $p = 0.001$) groups indicating that Fx was

able to better control cancerous cell (PA – 1 cell) growth than Tx at these concentrations. However, Tx performed better and suppressed the growth of PA – 1 cells more (attaining lower percent viability) than Fx at 10 μM (p = 0.005). This trend suggests that although at higher concentrations Tx may be more effective than Fx as there is a relative

decrease in the equivalent concentrations of the two drugs, they reach equal effectiveness initially, and finally, Fx attains a better ability to control the growth of PA – 1 cells. The effects of Fx and Tx were comparable at all other concentrations (all p > 0.05). (fig.3)

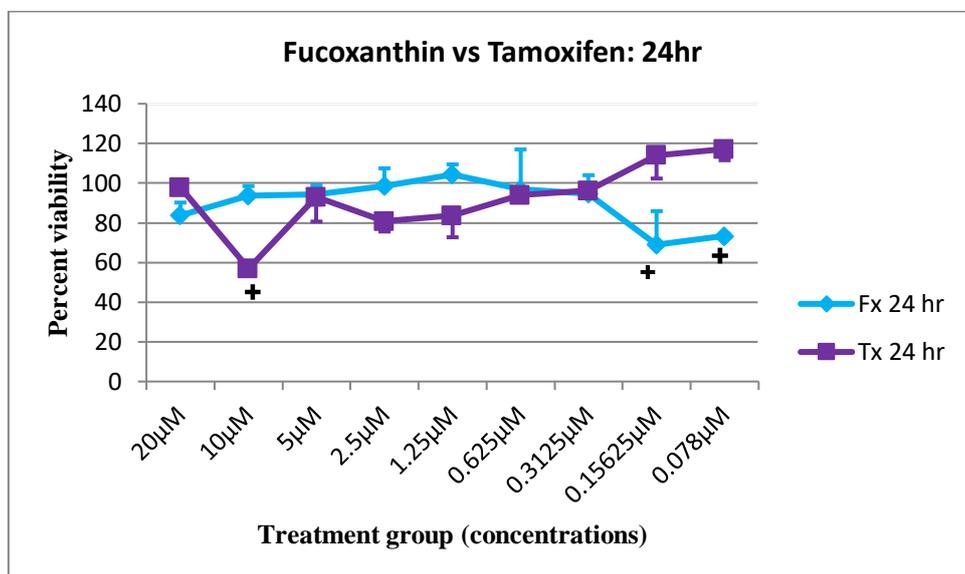


Fig.3: +Comparison between Fx and Tx at 24 hr, P< 0.05

Comparative effects of Fx and Tx on PA – 1 cells at 48 hrs

However, after 48 hr of incubation, no overall statistically significant interaction between the factors of drug types and treatment levels on percent viability was evident (F (9, 40) = 1.609, p= 0.146, partial η²= 0.266), although at two isolated concentrations the

drug treatments showed differences. Fucoxanthin was marginally better in reducing cell viability than Tx at a dose of 0.625 μM (p = 0.047) dose whereas Tx significantly bettered Fx at 5 μM (p = 0.015) dose. Taken together, it can be said that both drugs performed at comparable levels after 48 hr which was worse than after 24 hr of incubation. (fig.4)

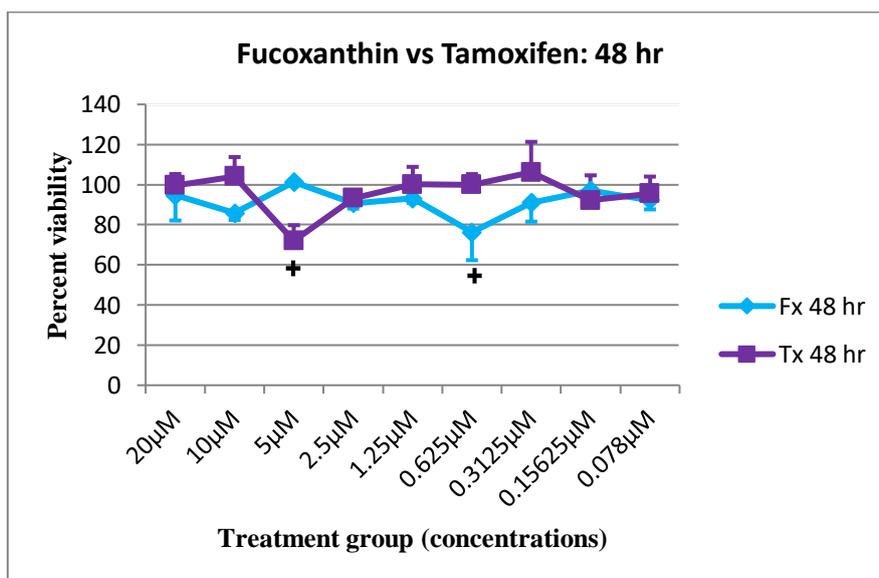


Fig.4: +Comparison between Fx and Tx at 48 hr, P< 0.05

IC₅₀(Half Maximal inhibitory concentration)

The anti-proliferative potency of both drugs on PA-1 cells was evaluated by determining IC₅₀ values. All

drugs were more potent on PA-1 cells (lower IC₅₀) after 24 hr incubation - Fx (IC₅₀ = 5.9μM, fig. 5A), Tx (IC₅₀ = 2.63μM, fig. 6A), when compared to their

respective 48 hr values ($IC_{50} = 18.21\mu M$, fig. 5B; $IC_{50} = 10.77\mu M$, fig.6B).

This suggests that lower concentrations of the drugs were able to reduce the viability of PA-1 cells after 24

hr incubation than 48 hr. Overall, luteinat 24 hr was found to be most potent against PA-1 cells in this experiment.

Fucoxanthin-PA1cells:24hr

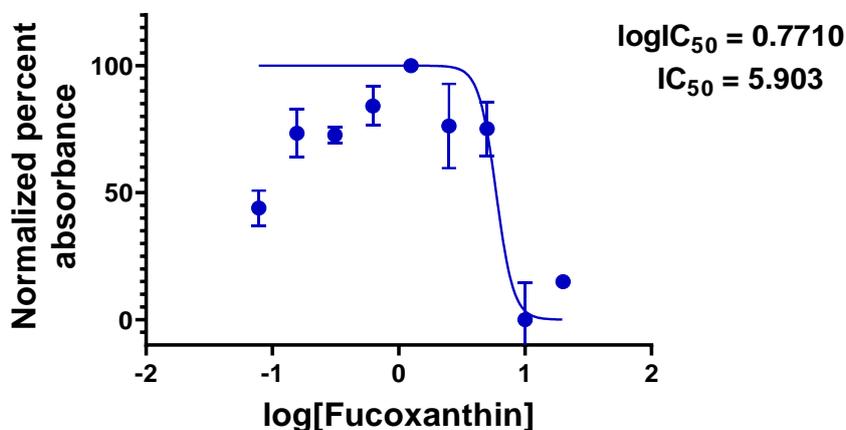


Fig. 5A

Tamoxifen - PA1cells: 24hr

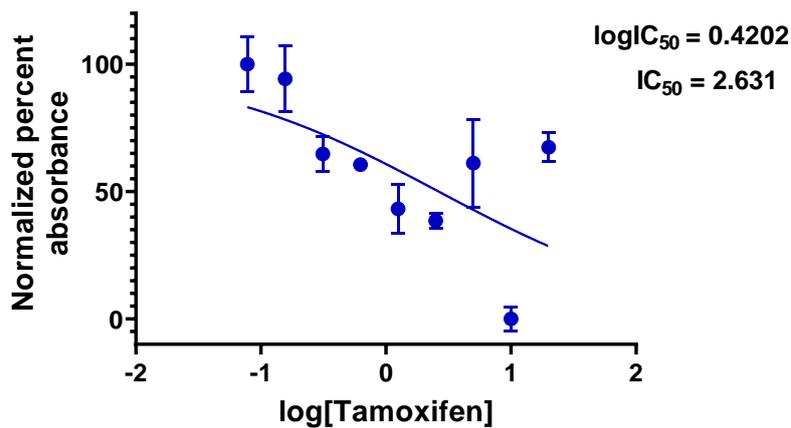


Fig. 6A

Fucoxanthin - PA1cells:48hr

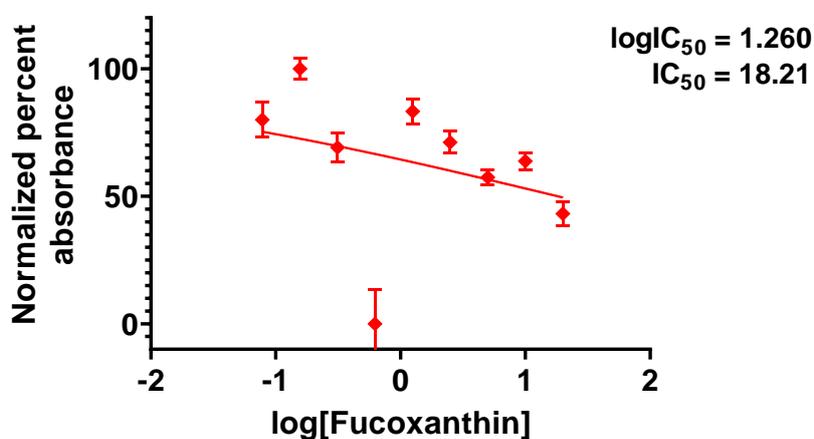


Fig. 5B

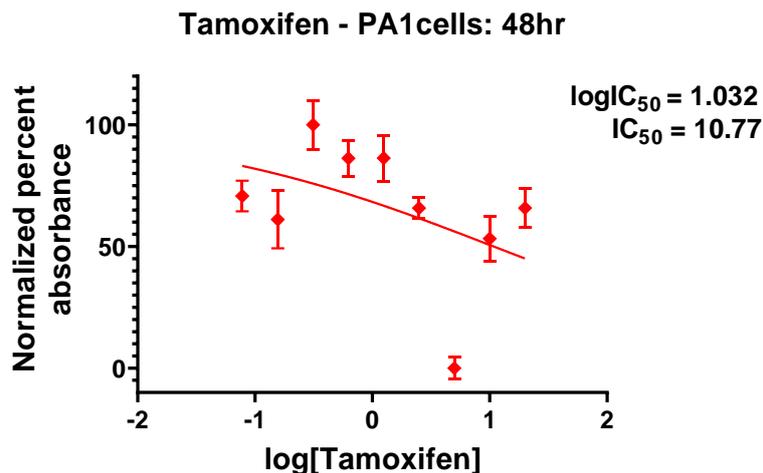


Fig. 6B

Apoptosis Assay

Annexin-V/FITC-PI staining coupled with flow-cytometry was used to mechanistically evaluate the concentration-dependent (three concentrations here) stages of cell death induced by different carotenoids.

Treatment of PA-1 cells with Fx

After treating PA-1 ovarian cancer cells with three different concentrations (in triplicates) of Fx, the distribution of cells in various stages of cell death as follows: (fig. 7, 7a, 7b, 7c, Table -1)

Fx treated PA-1 cells with three different concentrations (in triplicates)	Distribution of cells (%) in various stages of cell death			
	LL (Unstained cells)	UL (Early apoptosis)	UR (Late apoptosis)	LR (necrosis)
F1- 20µM	47.01±0.5	12.4±0.13	38.9±0.41	2.16±0.02
F2 -10µM	52.8±0.56	23.6±0.25	23.6±0.24	0.45±0.004
F3- 5µM	43.7±0.46	32.3±0.34	23.09±0.24	1.31±0.01

Table1: LL-lower left quadrant, UL-upper left, UR-upper right, LR-lower right

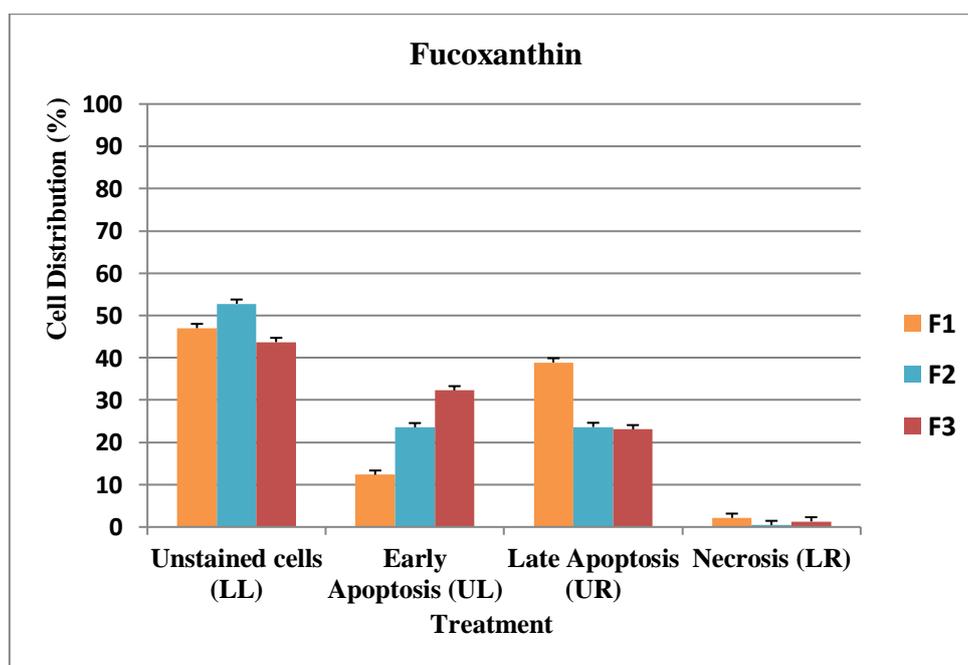


Fig. 7: Distribution of Fx treated PA-1 cells (in triplicates) in various stages of apoptosis at 24 hrs

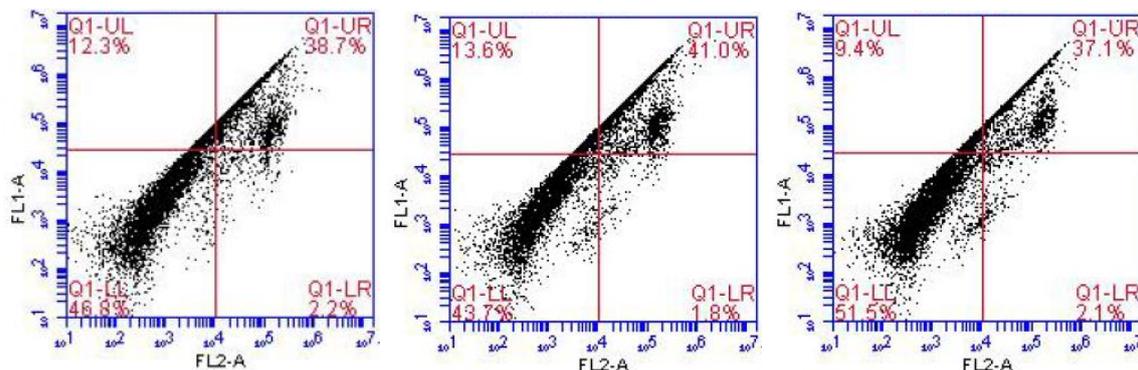


Fig.7a Fig.7bFig. 7c

Flow cytogram representing percentages of Fx treated PA-1 cells in various stages at 24 hrs: LL-unstained cells, UL- early apoptosis, UR- late apoptosis, LR- necrosis. PA-1 cells were treated with Fx (in triplicates); F1= 20µM (Fig. 7a), F2= 10µM (Fig. 7b), and F3= 5µM (Fig. 7c); Annexin V-FITC/PI staining

Treatment of PA-1 cells with Tx

After treating PA-1 cells with three different concentrations (in triplicates) of Tx, the distribution of cells in various stages of cell death as follows: (fig: 8, 8a, 8b, 8c, Table: 2)

Tx treated PA-1 cells with three different concentrations (in triplicates)	Distribution of cells (%) in various stages of cell death			
	LL(Unstained cells)	UL (Early apoptosis)	UR (Late apoptosis)	LR (necrosis)
T1- 20µM	51±0.53	15.34±0.16	34.02±0.36	0.08±0.0008
T2- 10µM	54.7±0.6	35.65±0.38	7.22±0.08	2.85±0.03
T3- 5µM	27.6±0.29	44.6±0.47	27.04±0.28	1.18±0.01

Table 2: LL-lower left quadrant, UL-upper left, UR-upper right, LR-lower right

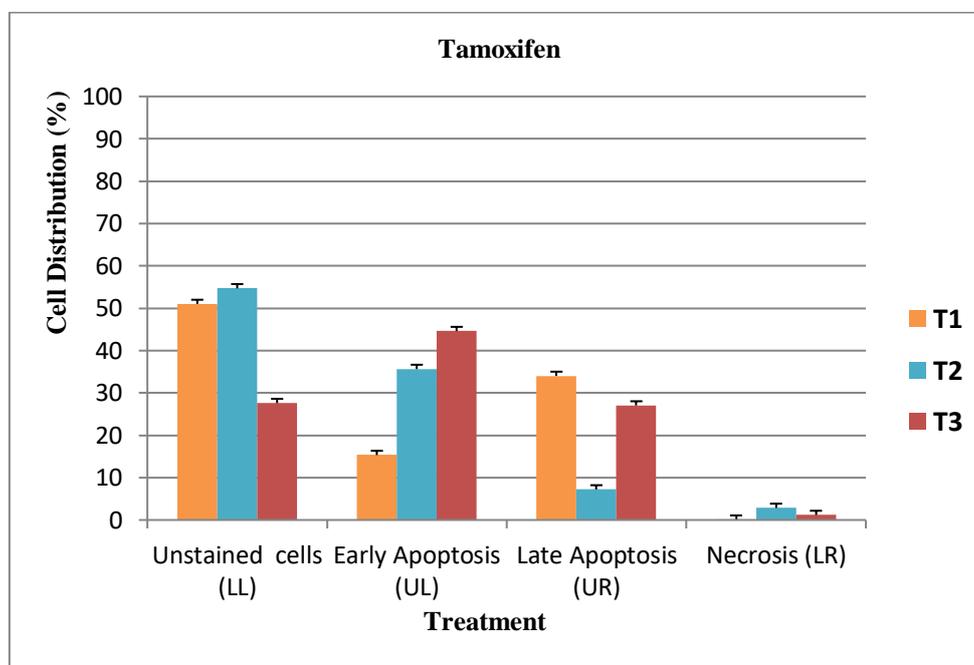


Fig 8: Distribution of Tx treated PA-1 cells (in triplicates)in various stages of apoptosis

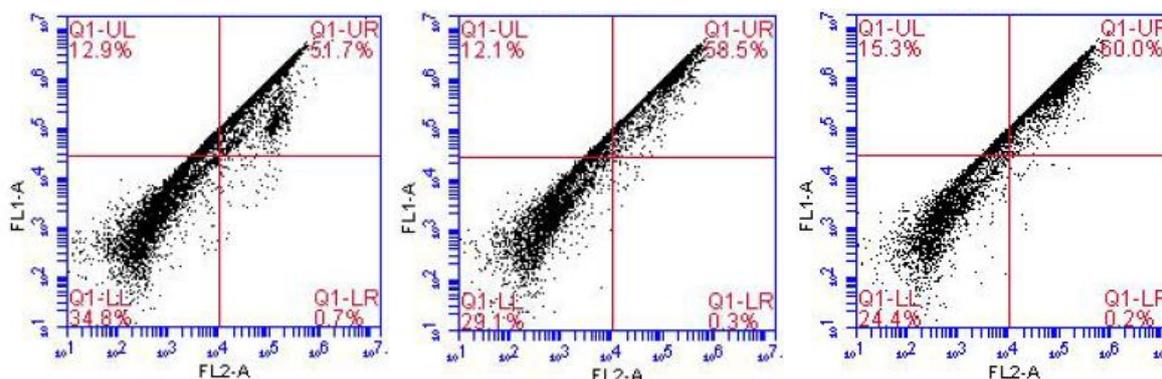


Fig: 8a **Fig.8bFig.8c**

Flow cytogram representing percentages of Tx treated PA-1 cells in various stages at 24 hrs: LL -unstained cells, UL- early apoptosis, UR- late apoptosis, LR- necrosis. HeLa cells were treated with Fx (in triplicates); F1= 20µM (**Fig. 8a**), F2= 10µM (**Fig. 8b**), and F3= 5µM (**Fig. 8c**); Annexin V-FITC/PI staining

Upon Post Hoc comparison, L2 of unstained cells was significantly lesser than T2 ($p=0.0449$); L2 of early apoptotic cells was significantly greater than T1 ($p=0.0327$), and L3 of necrotic cells was significantly higher than T1 ($p=0.0384$). Upon Post Hoc comparison, T2 of unstained cells was significantly higher as compared to T3 ($p=0.0080$); T1 of late apoptotic cells was significantly greater than T2 ($p=0.0327$), and T1 of necrotic cells was significantly lower than T2 ($p=0.0066$). All other concentrations were statistically insignificant.

DISCUSSION

We revealed the in-vitro anticancer efficacy of Fx on ovarian cancer PA-1 cells against Tx. In the present experiments, there was no statistically significant interaction between the factors of time points and treatment levels on the effect of percent viability by Fx at both 24 hrs and 48 hrs incubations. The mean percent viabilities of all drug concentrations were comparable to that of respective controls (fig. 1).

For Tx treatment, there was an overall statistically significant interaction between the factors of time points and treatment levels on the effect of percent viability. Specifically, the mean percent viabilities of cells at 24 hrs were significantly lesser than at 48 hrs with 10 µM ($p < 0.001$), 2.5 µM ($p = 0.023$), 1.25 µM ($p = 0.008$) and 0.3125 µM ($p = 0.023$) respectively, suggesting that 24 hrs incubation was better to reduce the growth of PA-1 cells (fig.2). However, 48 hrs incubation period did not show any significant effect of different concentrations on percent viability suggesting that 48 hrs incubation was not effective in controlling the growth of PA-1 cells in our experiment (fig. 2).

While the anti-cancer properties of carotenoid have been proven on various cancers by previous researchers, not many studies have been performed on ovarian cancer cells. Few plausible mechanisms causing the inhibition and apoptosis of ovarian cancer cells have been proposed. The involvement of the P13/AKT pathway was suggested in cervical cancer cell proliferation, cell cycle regulation, and apoptosis.

It serves a key role in the occurrence and development of a tumor.[14, 15]NF-kappa B is activated in many cancers, including inflammation, proliferation, and angiogenesis. It mediates the inhibition of apoptosis by biological signals and promotes the growth of tumor cells.[14]

Li et al. (2020) investigated the anticancer effects of Fx on A2780 ovarian cancer cells for 24 hrs. The cytotoxic efficacy of Fx against A2780 cells was determined using the MTT test. Fucoxanthin exhibited cytotoxic effects on A2780 cells at concentrations ranging from 5 to 100 µM. Treatment reduced cell viability by 80% relative to control cells. As a result, these findings demonstrated that Fx therapy could potentially promote cell death in A2780 cells. The nuclei were primarily broken into smaller fragments, implying the development of apoptotic bodies in Fx-treated cells. Fx (20 µM) treatment caused an increase in late apoptotic cells. The same treatment dramatically altered the mitochondrial membrane potential in A2780 cells. Fucoxanthin significantly boosted caspase 9 activation when compared to control A2780 cells as well as increased the expression of caspase 8 in A2780 cells. [16]

In our experiments, comparative analysis of Fx and Tx after 24 hrs of incubation showed a statistically significant interaction between the factors of drug types and treatment levels on the effect of percent viability. Fucoxanthin was able to better control cancerous cell growth than Tx at 0.15625 and 0.078µM. However, Tx suppressed cell growth effectively as compared to Fx at 10µM at 24 hrs (fig. 3). After 48 hrs of incubation, Fx was marginally better in reducing cell viability than Tx at a dose of 0.625 µM ($p = 0.047$) dose whereas Tx significantly bettered Fx at 5 µM ($p = 0.015$) dose (fig. 4). Taken together, it can be said that both drugs performed at comparable levels after 48 hrs which was worse than after 24 hrs of incubation.

We tested the anticancer effects of lower Fx concentrations at two time points (24 and 48 hrs) and found that the carotenoid inhibited cell growth at 0.15625 and 0.078µM as compared to the findings of

Li et al. where the higher Fx concentrations and 24 hrs post-incubation were more effective. They concluded that Fx potentially repressed the cancer cell proliferation, via inhibition of Akt/mTOR/S6K signaling pathway in ovarian cancer cells. Down-regulation of S6K resulting in the inhibition of Akt signaling cascade reveals affirmative feedback of Akt pathway regulation via S6K. Fucoxanthin-induced apoptosis, reduced cell proliferation, migration, and invasion; and revealed a potential mechanism of Fx-mediated Akt/mTOR suppression in human ovarian cancer cell line. [16]

A recent study examined the anticancer effects of Fx concentrations (50, 75, 100 μ M) on OVCAR-3 and A2780 for 48 hours. Fucoxanthin inhibited the STAT3/c-Myc signaling pathway in ovarian cancer, reducing cell proliferation, metastasis, and glycolysis.[17]

CONCLUSION

Few studies in cell models have revealed the antitumor effects of Fx and their mechanisms of action. According to previous literature, Fx can suppress the proliferation of cancer cells, through inhibiting Akt/mTOR/S6K and inhibiting the STAT3/c-Myc signaling pathway, concluding that Fx can be a potential anticancer agent for ovarian cancer cells. The ability of fucoxanthin to regulate these signaling pathways contributes to its anti-proliferative and pro-apoptotic effects on ovarian cancer cells. To our knowledge, the anticancer effects of Fx and lutein on ovarian cancer cells have not been fully documented, which is what the present study contributes to. A major limitation of the present study is its inability to provide mechanistic insight into the subcellular activity of carotenoids. Furthermore, the mechanisms and pathways that contribute to cell cycle regulation, cell proliferation inhibition, and apoptosis remain to be elucidated.

Fx exhibits favorable safety profiles and low toxicity levels in normal cells, making it an attractive candidate for potential therapeutic use. Its natural origin from seaweed and its relatively low side effects compared to conventional chemotherapy further enhance its appeal as a potential adjunct or alternative treatment for ovarian cancer. Despite the promising preclinical studies highlighting the anticancer effects of Fx on ovarian cancer cells, further research is essential to fully understand its mechanisms of action, optimal dosage, bioavailability, and potential synergistic effects with other therapeutic agents. Clinical trials evaluating the efficacy of Fx in ovarian cancer patients are warranted to validate its therapeutic potential and safety in a clinical setting.

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