Pharmaceutical Sciences, 2023, 29(3), 320-327
doi:10.34172/PS.2022.44
https://ps.tbzmed.ac.ir/

Research Article

Fucoxanthin Inhibits the Proliferation of ABCC2-Over Expressing Cisplatin-Resistance Ovarian Cancer Cells via Inducing Apoptosis

Fatemeh Valinezhad Sani[1], Safa Kamalian[2], Hakimeh Abdi[2], Shiva Ghofrani[2], Arad Boustan[3], Fatemeh Mosaffa[4*]

[1]*Department of Laboratory Sciences, School of Paramedical and Rehabilitation Sciences, Mashhad University of Medical Sciences, Mashhad, Iran.*
[2]*Department of Pharmaceutical Biotechnology, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran.*
[3]*Department of Medical Biotechnology and Nanotechnology, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.*
[4]*Biotechnology Research Center, Pharmaceutical Technology Institute, Mashhad University of Medical Sciences, Mashhad, Iran.*

Article Info

Article History:
Received: 8 Oct 2022
Accepted: 11 Nov 2022
ePublished: 24 Nov 2022

Keywords:
-Cisplatin
-Cytotoxicity
-Fucoxanthin
-Multidrug resistance (MDR)
-Ovarian cancer

Abstract

Background: The development of multidrug resistance (MDR) is a major barrier to achieving effective chemotherapy in cancer. Studies have shown that epithelial ovarian cancer initially responds to platinum-based therapy, however, the recurrent type is often resistant to treatment and is associated with high mortality. Fucoxanthin, a natural component found in marine algae, possesses various pharmacologic properties. This study evaluated the cytotoxicity and resistance reversal activity of fucoxanthin on multidrug resistance-associated protein 2 (MRP2)-overexpressing, cisplatin-resistant ovarian cancer cells (A2780RCIS) and their parental cells (A2780).

Methods: Cell viability was evaluated in the presence of different concentrations of fucoxanthin or cisplatin or fucoxanthin/cisplatin combination using the MTT assay. Propidium iodide staining and subG1 analysis were used to evaluate fucoxanthin potential for cell cycle modification and apoptosis induction in cancer cell lines.

Results: The results showed that fucoxanthin was able to cause similar toxicity in both cell lines via apoptosis induction. Co-treatment of cells with cisplatin (3.125 to 100 µM) and nontoxic concentrations of fucoxanthin (1 and 2.5 µM) did not reverse resistance to cisplatin in A2780RCIS cells.

Conclusion: Although fucoxanthin was not able to modify cisplatin resistance in ovarian cancer cells, it was equally effective in inducing apoptosis and death in both A2780 and A2780RCIS cells, indicating it is not an MRP2 substrate.

Introduction

Among gynecologic cancers, ovarian carcinoma causes the highest mortality. Because of the lack of effective screening methods and explicit symptoms associated with early-stage disease, most patients with ovarian cancer are diagnosed at late stages. These patients are treated by surgical excision of the cancer followed by a platinum/taxane combination chemotherapy. Although, this first-line treatment regime is effective, in up to 75% of ovarian cancer patients, recurrence occurs which is followed by acquired drug resistance.[1] Therefore, the development of new therapies, drug-resistance modulators or chemosensitizers with low toxicity and minimum undesirable side effects has gained clinical interest in recent years.[2]

Fucoxanthin, is an orange-colored pigment from marine algae that contributes 10% of the total carotenoids in nature.[3] It has been used for centuries as a traditional supplement and fucoxanthin-rich brown seaweed is used as food in East Asian countries diets. Recent reports have shown pharmacological potential of fucoxanthin including anticancer, anti-inflammatory, antioxidant, neuroprotective, anti-hyper-lipidemia and anti-obesity activities.[4] Different potential mechanisms have been reported for the anti-carcinogenic effects of fucoxanthin such as DNA fragmentation by activation of endogenous nucleases,[5] suppression of cyclin D expression and proteosomal degradation and activation of the caspase pathway.[6] Liu *et al.*[7] have reported that the anti-cancer properties of fucoxanthin are related to increased intracellular calcium levels through enhanced expression of connexin genes and gap junction intracellular communication. Recently, it has been reported that the combination of fucoxanthin with other chemotherapeutic drugs was beneficial in breast and lung cancer therapies.[8,9] In the present study for the first time we examined the likely benefits of fucoxanthin application in

*Corresponding Author: Fatemeh Mosaffa, E-mail: Mosaffaf@mums.ac.ir

ovarian cancer therapy and overcoming drug resistance in this cancer using cell line models. We evaluated the toxicity and cisplatin-resistance reversal activity of fucoxanthin in A2780RCIS cells already proved to be resistant against cisplatin via overexpressing MRP2 (ABCC2), a well-known transmembrane ABC efflux transporter which pumps cisplatin out of the cells.[10,11] We also compared the effects of fucoxanthin in the cisplatin-resistant cells with cisplatin-sensitive ovarian cancer cells, A2780.

Materials and Methods

Cell lines and reagents

Drug-sensitive and resistant human ovarian cancer cell lines, A2780 and A2780RCIS were kindly provided by Dr Hermann Lage, Institute of Pathology, Charite Hospital, Berlin, Germany. Both cell lines were cultured in RPMI-1640 medium supplemented with 10% of fetal bovine serum, 1% penicillin (100 units/ml), and streptomycin (100 mg/ml) at 37^{0}C in a humidified atmosphere with 5% (v/v) CO_2. Fucoxanthin and cisplatin were purchased from Sigma (USA) and Ebewe Pharma (Austria) respectively. 3-(4, 5-dimethylthiazol-2-yl)-2, 5- diphenyltetrazolium bromide (MTT) was provided from Alfa Aesar (USA). Propidium iodide (PI) was obtained from Sigma (USA). All other chemicals and reagents obtained from commercial sources were of analytical or cell culture grade.

Cell cytotoxicity assay

The cytotoxic effect of fucoxanthin was measured by MTT assay. A2780 and A2780RCIS cells were seeded in 96-well dishes (5.0×10^3 cells/well). After 24 h incubation, the cells were exposed to different concentrations of fucoxanthin (0, 2.5, 10, 20, 60, 80 µM) for 48 and 72 h. After treatment, the cells were incubated with 10 µl MTT (5 mg/ml) for 4 h, then the medium was discarded and formazan crystals were solubilized with DMSO (100 µl/well). The spectrophotometric absorbance was measured at 570 and 630 nm (reference wavelength) using a microplate reader, and the percentage of viable cells was calculated by the ratio of OD test/OD control.

Evaluation of cisplatin-resistance reversal activity

To assess the cisplatin-resistance reversal effect of fucoxanthin, A2780RCIS cells were seeded into 96-well plates, then treated with different concentrations of cisplatin (0, 3.125, 6.25, 12.5, 25, 50, and 100 µM) alone or in the presence of non-toxic concentrations of fucoxanthin (1 or 2.5 µM) for 48 and 72 h, and the cell viability was determined via MTT assay as previously described.

Sub-G1 and cell cycle distribution analysis

To explore the mechanism of cell death caused by fucoxanthin, propidium iodide staining method was applied. Ovarian cancer cell lines at a density of 3.5×10^5 cells/ml were cultured in 6-well plates overnight, then exposed to fucoxanthin (20 µM) for 48 h. Then cells were harvested and centrifuged at 400×g for 5 min. The pellets were resuspended in 400 µl PI staining solution (50 µg/ml) containing sodium citrate (0.1 %), Triton X-100 (0.1%) and RNase A (0.1 mg/ml) and then incubated for 30 min in the dark. Following incubation, the cells were centrifuged and washed twice with ice-cold phosphate buffered saline (PBS) and resuspended in ice-cold PBS. Finally, the cells were analyzed by flow cytometry using FL2 channel (BD FACS Calibur, USA). The percentage of cells in different phases of the cell cycle and sub-G1 (as a hallmark of DNA fragmentation and apoptosis induction) was calculated with Flowjo7.6.1 software.

Microscopic examination of the cell morphologies

A2780 and A2780RCIS were seeded at a density of 3.5×10^5 cells per well of 6-well plates. Following an overnight incubation, the cells were treated with fucoxanthin (20 µM) or cisplatin (3.9 µM) for 48 h and microscopic images were captured from different treatments by a CCD camera connected to an inverted phase-contrast microscope (INVERSO-TC100, Medline Scientific Limited, United Kingdom) for visualization of the appearance changes of the cells following the treatments.

Statistical analysis

All results were expressed as mean ± standard deviation (SD) from three independent experiments. In order to compare the data from different groups, Student's t-test or one-way ANOVA with post-hoc Dunnett's test were applied using Prism version 8 software. P value < 0.05 was considered significant.

Results

The effect of fucoxanthin on the cell viability of A2780 and A2780RCIS cancer cell lines

To evaluate the fucoxanthin effects on the viability of A2780 and A2780RCIS cancer cells, the cells were incubated with different concentrations of fucoxanthin for 48 and 72 h, and then an MTT assay was performed. As shown in Figure 1, fucoxanthin resulted in a concentration-dependent inhibitory effect on both cisplatin sensitive and resistant ovarian cancer cells. There was no significant difference between IC_{50} of fucoxanthin at 72 h incubation times in each cell line and fucoxanthin toxicity toward cisplatin-resistant A2780RCIS cells was nearly equal to its lethal effects on A2780 cisplatin-sensitive cells (Table 1). Fucoxanthin at the concentration of 2.5 µM had no significant effect on the proliferation of A2780 and A2780RCIS cells. Therefore, 1 and 2.5 µM concentrations of fucoxanthin were selected for cisplatin-resistance reversal experiment.

The effect of fucoxanthin on cisplatin-resistance phenotype in A2780RCIS cells

In order to assess the cisplatin-resistance reversal effect of fucoxanthin, first the sensitivity to cisplatin was compared between two cell lines. As shown in Figure 2, cisplatin significantly decreased the proliferation rate of A2780 and A2780RCIS cells in a time and dose-dependent manner.

Table 1. Fucoxanthin IC_{50}s in A2780 and A2780RCIS ovarian cancer cell lines.

Cell line	Fucoxanthin IC_{50} (μM)- 48 h	Fucoxanthin IC_{50} (μM)- 48 h
A2780	11.00 ± 0.53	10.94 ± 0.53
A2780RCIS	13.13 ± 1.06*	11.57 ± 0.50*

The cells were treated with different concentrations of fucoxanthin for 48 and 72 h, then the sensitivity of ovarian cancer cell lines to cisplatin was measured by MTT assay and the IC50 values of fucoxanthin were determined. Each experiment was performed three times and the results were shown as mean ± SD. **P < 0.05* compared to sensitive cells incubated with fucoxanthin for 48 h (unpaired Students't test).

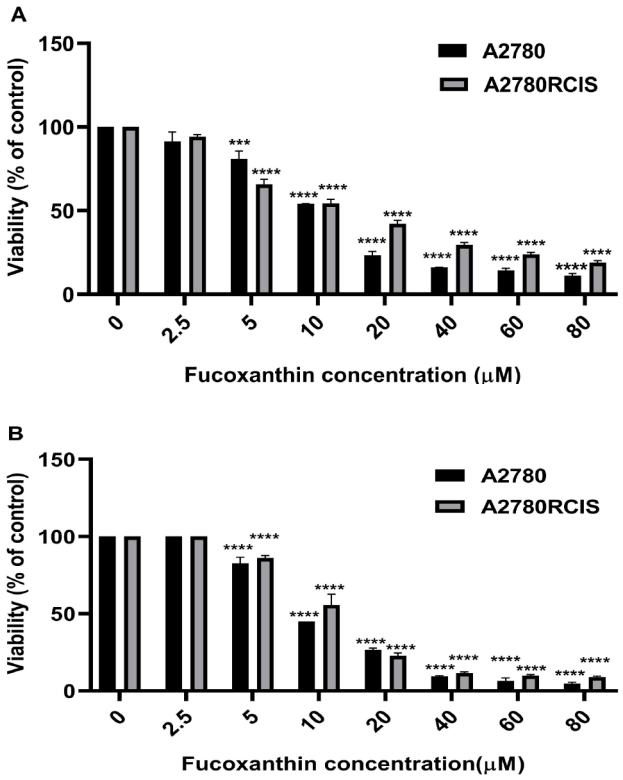

Figure 1. The effects of fucoxanthin on the viability of A2780 and A2780RCIS cells. The cells were treated with different concentration of fucoxanthin for 48 (A) and 72 h (B), then cell viability percentage was measured using MTT assay. Each experiment was carried out at least in triplicate and the results were presented as mean ± S.D. ****P < 0.001, ****P < 0.0001* compared to untreated control cells (one-way ANOVA followed by post-hoc Dunnett's test).

Cisplatin IC_{50} values obtained for two cell lines confirmed that it was much more toxic against parental A2780 cells in comparison with the resistant cell line A2780RCIS following both 48 and 72 h incubation periods (Table 2). Furthermore, the cytotoxicity of different concentrations of cisplatin in the presence of a non-toxic concentration of fucoxanthin (1 or 2.5 μM) was assessed in both cell lines following 48 and 72 - cotreatments. Our results showed that non-toxic concentration of fucoxanthin could not change the sensitivity of both cell lines toward cisplatin (Figure 3).

The apoptosis-inducing effect of fucoxanthin on A2780 and A2780RCIS cancer cell lines

The apoptosis-inducing effect of fucoxanthin was evaluated using PI staining of DNA content and flow cytometry (Figures 4 and 5). Internucleosomal degradation of DNA, resulted from the activation of endogenous endonuclease, is one feature that happens during apoptosis and cells with the fragmented DNA appear in the sub-G1 area of PI florescent histograms. While cisplatin treatment (3.9 μM) resulted in about 20% significant increase in the apoptotic (sub-G1) cell count in A2780 cells (Figures 4B and 5A)

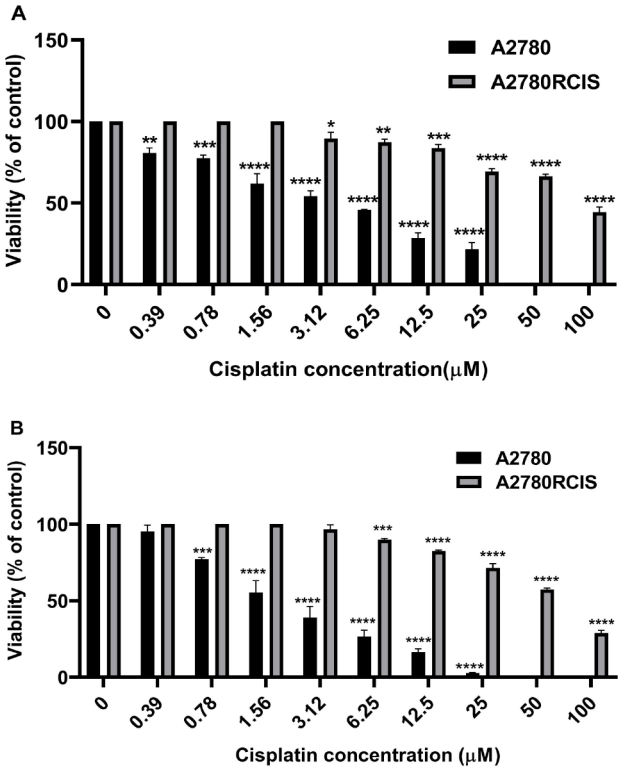

Figure 2. The effects of cisplatin on the viability of A2780 and A2780RCIS cell lines. The cells were treated with different concentrations of cisplatin for 48 (A) and 72 h (B), then the sensitivity of ovarian cancer cell lines to cisplatin was measured by MTT assay. Each experiment was performed three times and the results were shown as mean ± SD. **P < 0.05*, ***P < 0.01*, ****P < 0.001*, *****P < 0.0001* compared to control groups *(*one-way ANOVA followed by post-hoc Dunnett's test).

Table 2. Cisplatin cytotoxicity in A2780 and A2780RCIS ovarian cancer cell lines.

Cell line	Cisplatin IC_{50} (μM)- 48 h	Cisplatin IC_{50} (μM)- 72 h
A2780	3.97 ± 0.56	2.51 ± 0.43#
A2780RCIS	88.93 ± 4.90****	63.76 ± 2.07**** ##

Cells were treated with different concentrations of cisplatin for 48 and 72 h, then the sensitivity of ovarian cancer cell lines to cisplatin was measured by MTT assay. The IC_{50} values of cisplatin were determined. Each experiment was performed three times and the results were shown as mean ± SD. *****P < 0.0001* compared to A2780 cells incubated with cisplatin for 48 h or 72 h, *# P < 0.05 and ## P < 0.01* compared to the same cell line treated with cisplatin for 48 h (unpaired Students' t-test).

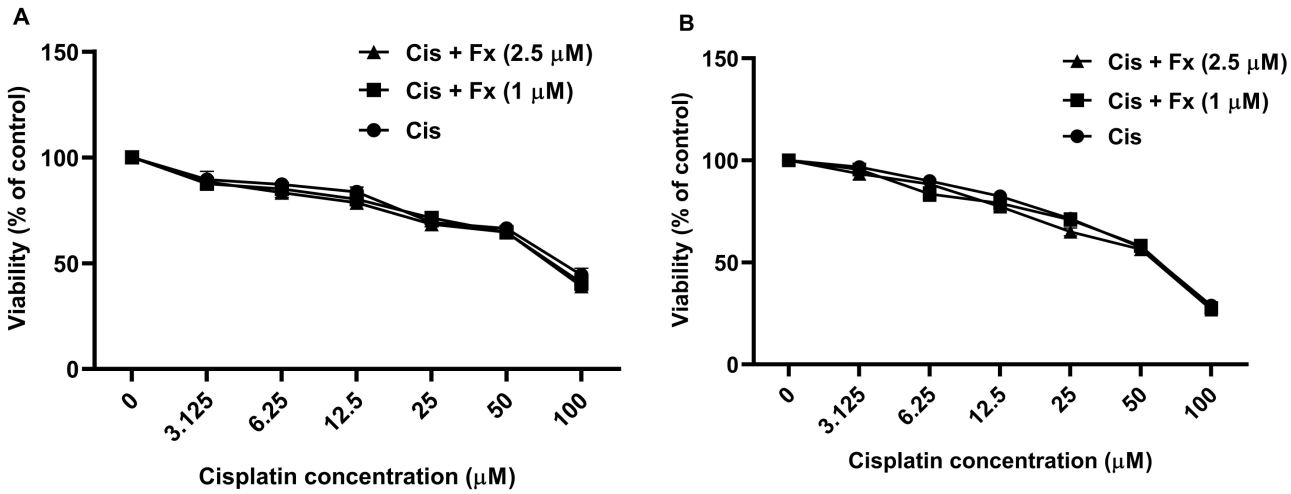

Figure 3. The effects of fucoxanthin on the toxicity of cisplatin in A2780RCIS cells. The cells were treated for 48 (A) and 72 h (B) with different concentrations of cisplatin in absence or presence of fucoxanthin (1 and 2.5 μM). Each experiment was performed three times and the results were shown as mean ± SD. There were no significant differences between viability of cisplatin treated and cisplatin + fucoxanthin cotreated cells at all tested concentrations of cisplatin (one-way ANOVA followed by post-hoc Dunnett's test). Cis (Cisplatin), Fx (Fucoxanthin).

compared to untreated ones (Figures 4A and 5A), at the same concentration it was not able to cause any significant change in apoptotic cell percentage in A2780RCIS cells (Figures 4E and 5B) compared to control cells (Figures 4D and 5B) which was in conformity with the MTT viability assay results. In contrast, when A2780 and A2780RCIS cells were treated with 20 μM fucoxanthin for 48 h, the majority of cells (about 68 % and 97 %, respectively) of both cell lines became apoptotic and had fragmented DNA (Figures 4C, 4F, 5A, and 5B).

The effects of fucoxanthin on cell cycle progression

The effects of fucoxanthin and cisplatin on cell cycle distribution of A2780 and A2780RCIS cells were assessed by PI staining and flow cytometry. As shown in Figure 5, 48 h-treatment with cisplatin (3.9 μM) caused a significant G2 arrest in A2780 cell (Figure 5A), but not in resistant A2780RCIS cells (Figure 5B) compared to the controls. Following 48 h treatment with fucoxanthin (20 μM) no significant changes were observed in the distribution of A2780 cells in the cell cycle phases compared with

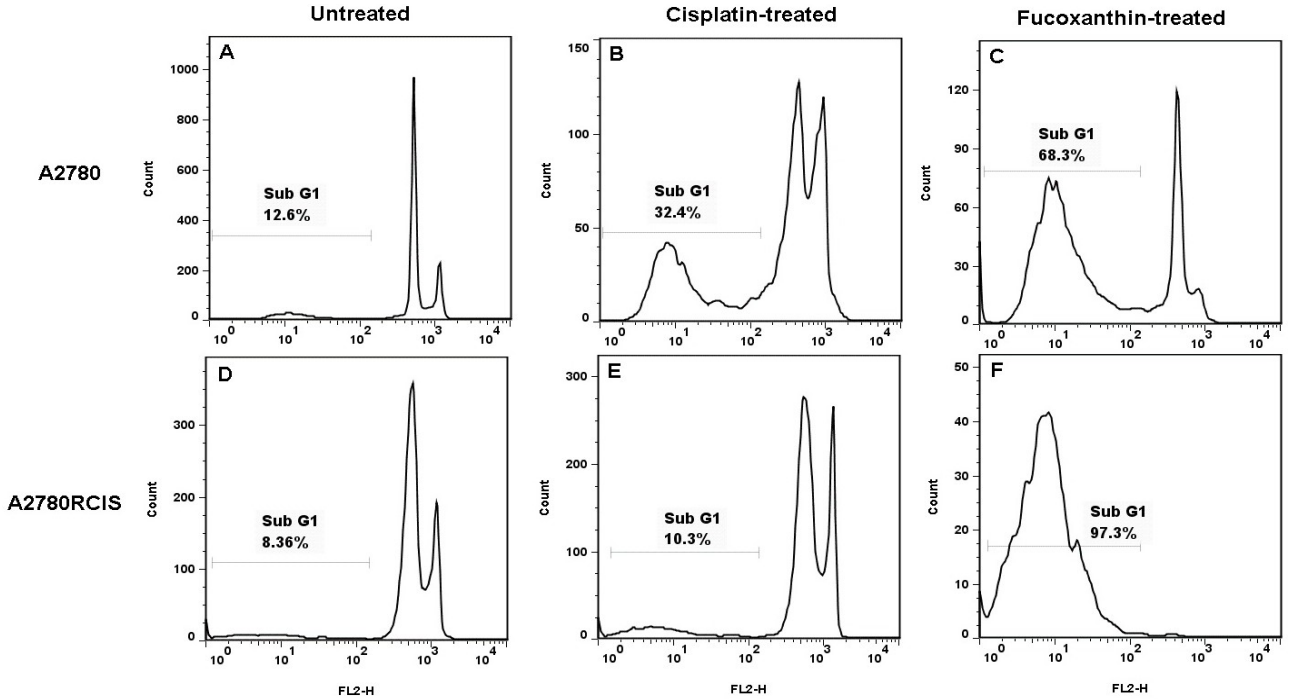

Figure 4. Evaluation of apoptosis induction using PI staining method and flow cytometry. The A2780 (A) and A2780RCIS (B) cells were treated with Cisplatin (3.9 μM) or fucoxanthin (20 μM) for 48 h. The sub-G1 peak, as an indicative of apoptotic cells, was analyzed and compared to untreated cells using flow cytometry. The histograms are representative of three independent experiments.

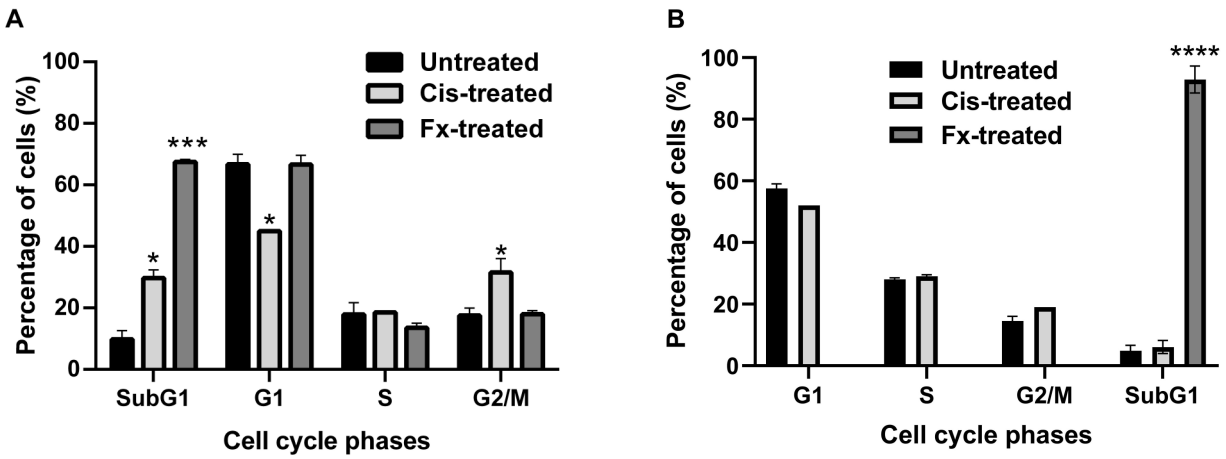

Figure 5. Cell cycle distributions of fucoxanthin or cisplatin-treated ovarian cancer cells measured with the flow cytometry analysis. A2780 (A) and A2780RCIS (B) cells were either untreated or treated with fucoxanthin (20 μM) or cisplatin (3.9 μM) for 48 h, then stained with PI, and analyzed by flow cytometry. The proportion of cells at each phase of cell cycle was presented as percentage of cells in the total cell cycle (cells in G1+S+G2/M). The proportion of sub-G 1 cells was also presented as percentage of the entire cell experiments. **P < 0.05, ***P < 0.001, ****P < 0.0001* compared to untreated control cells (one-way ANOVA followed by post-hoc Dunnett's test).

untreated ones (Figure 5A) and almost all of A2780RCIS cells had fragmented DNA (Figure 5B).

The effects of fucoxanthin treatment on the morphology of the cells

The exposure of A2780 and A2780RCIS cells to 20 μM fucoxanthin for 48 h clearly induced the morphological changes indicative of apoptotic cell death in both cell lines including rounding up, reduction of cell size, nuclear condensation and fragmentation and formation of apoptotic bodies (Figure 6). The same morphological changes were observed in A2780 parental cells exposed to cisplatin (3.9 μM) but not in their cisplatin-resistant derivatives, A2780RCIS cells.

Discussion

Ovarian cancer is one of the most common gynecologic cancers with increasing mortality rates in most parts of the world.[12] Although, cisplatin has been widely used as a first-line treatment for ovarian cancers, unfortunately, the phenomenon of resistance to it is one of the main obstacles which leads to the ineffectiveness of platinum-based chemotherapy treatments. Efforts to find effective chemosensitizers or new therapies against drug-resistance ovarian cancer cells, especially natural compounds with fewer side effects for the human body can lead to the development of more successful therapies in the future.

Fucoxanthin is a well-known natural dietary carotenoid commonly found in seaweeds and diatoms. There are many pharmacological evidence about its health-promoting effects including anti-cancer[13] and anti-inflammatory properties,[14] its protective effects against doxorubicin-induced cardiotoxicity,[15] diabetic nephropathy,[16] alcoholic liver injury[17] and other metabolic diseases. The multi-potent health effects of fucoxanthin are attributed to its unique chemical structure including an allenic bond, an acetyl group, a conjugated carbonyl group, and 5,6-monoepoxide.[4] The long traditional consumption

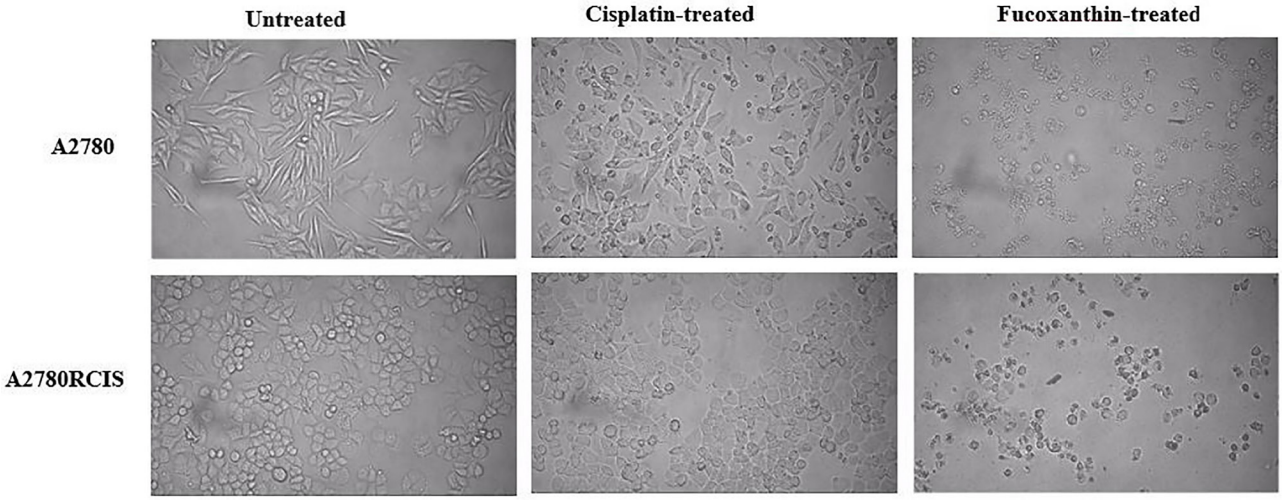

Figure 6. The effect of fucoxanthin on A2780 and A2780RCIS cell proliferation. The cells treated with fucoxanthin (20 μM) or cisplatin (3.9 μM) for 48 h and the images were captured by an inverted phase-contrast microscope (×200).

of fucoxanthin supports its safety. Furthermore, it has been shown that fucoxanthin did not cause any toxicity or adverse effects in different study models and can be considered as a safe pharmaceutical component.[18]

In the present study, we evaluated the effect of fucoxanthin on the survival of cisplatin sensitive and resistant ovarian cancer cells as well as its effect on reducing the resistance to cisplatin in MRP2 overexpressing, cisplatin-resistant ovarian cancer cells. Following the treatment of the cells by cisplatin, the results obtained by MTT viability assay, PI staining of DNA content and microscopic examination all validated the resistance of A2780RCIS cells to cisplatin compared to its parental A2780 cells. The results of cytotoxicity assay also indicated that fucoxanthin, when used alone for the treatment of the cells, has a concentration-dependent cytotoxicity in both cell lines. Unlike cisplatin, fucoxanthin showed almost similar toxicity in both sensitive and resistant cells, which reveals that this compound is not a substrate for the MRP2 pump and therefore, has the potential to induce death in cisplatin-resistant ovarian cancer cells. Other researchers previously reported that P-gp (ABCB1)-overexpressing breast, liver, and ovarian cancer cell lines, MCF-7/ADR, Hep-G2/ADR and SKOV-3/ADR, which were resistant to doxorubicin showed the same degree of resistance toward fucoxanthin compared to their parental cells, MCF-7, Hep-G2 and SKOV-3 cells. They concluded that fucoxanthin is a substrate of P-gp transporter which leads to its efflux by the pump.[19] We also found that fucoxanthin at the nontoxic concentrations of 1 and 2.5 µM could not increase the sensitivity of A2780RCIS cells to cisplatin at 48 or 72 h. Combination therapy which implied that these concentrations of fucoxanthin is not an inhibitor or modulator of MRP2 activity. In the study conducted on MCF-7/ADR, Hep-G2/ADR and SKOV-3/ADR cells, fucoxanthin at nontoxic concentration enhanced doxorubicin cytotoxicity in these cells. In addition, it increased the accumulation of the P-gp substrates, doxorubicin and rhodamine123, in the resistant cells which was suggestive that it is an inhibitor or modulator of the function of P-gp.[19] In the next step, to investigate the mechanism of fucoxanthin cytotoxicity, ovarian cancer cells treated with 20 µM fucoxanthin were evaluated by PI staining followed by flow cytometry and microscopic examination. The results showed that unlike cisplatin that was able to induce G2/M arrest in sensitive ovarian cancer cells, fucoxanthin-induced apoptosis in these cells without affecting cell proportions in different phases of the cell cycle. The key cytotoxicity mechanism of cisplatin is the formation of DNA intra- and interstrand cross-links that causes bulky DNA damage. As an effort to repair the cisplatin-induced DNA damage a checkpoint response prevents the cells from cell-cycle progression and they arrest in the G2M phase and finally die via apoptosis.[20] As fucoxanthin did not cause any cell cycle arrest in the ovarian cancer cells, it probably activates an apoptotic pathway other than the pathway activated by DNA damage responses in these cells. In previous studies on the other cancer types and cell lines conducted with the aim of cell death characterization induced by fucoxanthin, apoptosis was frequently demonstrated to be the responsible mechanism for cell toxicity, however, various pathways were reported to be involved in the induction of apoptosis.[5,6] Fucoxanthin is a well-known antioxidant that is also shown to be pro-oxidant. Reactive oxygen species (ROS) ROS are well recognized as mediators of DNA damage. In hepatic BNL CL.2 cells, 6-h fucoxanthin (5 µM) treatment augmented reactive oxygen species (ROS) levels in the cells, which returned to the control level at 12 h of incubation.[21] In another study on human promyelocytic leukemia cells, HL-60, 24 h-fucoxanthin (10 µM) treatment did not affect the ROS level at all. The authors suggested that ROS was not involved in the main pathway of apoptosis induced by fucoxanthin in these cells. This finding was in correlation with our results as we also noticed no detectable alteration in the cell cycle distribution (naturally accompanied by DNA damage response) in the 48 h-fucoxanthin-treated ovarian cancer cells. As fucoxanthin caused the loss of mitochondrial membrane potential at an early stage of treatment, they also concluded that fucoxanthin leads to apoptosis via the mitochondria in a manner different from oxidative stress. Fucoxanthin also induced cleavages of procaspase-3 and rabbit anti-poly (ADP-ribose) polymerase (PARP) which demonstrates the apoptosis induced by fucoxanthin was caspase-3 dependent.[22] There are also other studies which reported the main role of caspase-3 in fucoxanthin-induced apoptosis in the human cervical cancer cells (Hela),[23] human bladder cancer cells (EJ-1 and T24),[24] osteosarcoma cells (Saos-2)[25] and endometrial cancer cells (HEC-1A).[26]

Conclusion

Although fucoxanthin was not able to modify cisplatin resistance in A2780RCIS ovarian cancer cells, it was highly effective in inducing apoptosis and death in both cisplatin sensitive and MRP2 overexpressing resistant ovarian cancer cells which demonstrates it is not an MRP2 substrate. To determine whether any clinical benefits exist for the administration of fucoxanthin in treating cisplatin-resistant ovarian cancers additional detailed investigations including animal studies in xenograft cancer models are required.

Acknowledgments

The authors gratefully acknowledge of the Vice-Chancellor of Research in Mashhad University of Medical Sciences, Mashhad, Iran (Grant number 980871) for approval and financial support of this research.

Author Contribution

Fatemeh Valinezhad Sani: Investigation, Writing – Original Draft. Safa Kamalian: Investigation, Formal analysis. Hakimeh Abdi: Methodology. Shiva Ghofrani: Methodology, Arad Boustan: Validation. Fatemeh Mosaffa:

Conceptualization, Funding Acquisition, Writing - Review & Editing.

Conflict of Interest

The authors report no conflicts of interest.

References

1. Agarwal R, Kaye SB. Ovarian cancer: strategies for overcoming resistance to chemotherapy. Nat Rev Cancer. 2003;3(7):502-16. doi:10.1038/nrc1123
2. Thomas H, Coley HM. Overcoming multidrug resistance in cancer: an update on the clinical strategy of inhibiting p-glycoprotein. Cancer Control. 2003;10(2):159-65. doi:10.1177/107327480301000207
3. Khaw YS, Yusoff FM, Tan HT, Noor Mazli NAI, Nazarudin MF, Shaharuddin NA, et al. The critical studies of fucoxanthin research trends from 1928 to June 2021: A bibliometric review. Mar Drugs. 2021;19(11):606. doi:10.3390/md19110606
4. Mohibbullah M, Haque MN, Sohag AAM, Hossain MT, Zahan MS, Uddin MJ, et al. A systematic review on marine algae-derived fucoxanthin: an update of pharmacological insights. Mar Drugs. 2022;20(5):279. doi:10.3390/md20050279
5. Hosokawa M, Kudo M, Maeda H, Kohno H, Tanaka T, Miyashita K. Fucoxanthin induces apoptosis and enhances the antiproliferative effect of the PPARgamma ligand, troglitazone, on colon cancer cells. Biochim Biophys Acta Bioenerg. 2004;1675:113-9. doi:10.1016/j.bbagen.2004.08.012
6. Kumar SR, Hosokawa M, Miyashita K. Fucoxanthin: a marine carotenoid exerting anti-cancer effects by affecting multiple mechanisms. Mar Drugs. 2013;11:5130-47. doi:10.3390/md11125130
7. Liu CL, Huang YS, Hosokawa M, Miyashita K, Hu ML. Inhibition of proliferation of a hepatoma cell line by fucoxanthin in relation to cell cycle arrest and enhanced gap junctional intercellular communication. Chem Biol Interact. 2009;182:165-72. doi:10.1016/j.cbi.2009.08.017
8. Malhão F, Macedo AC, Costa C, Rocha E, Ramos AA. Fucoxanthin holds potential to become a drug adjuvant in breast cancer treatment: evidence from 2D and 3D cell cultures. Molecules. 2021;26(14):4288. doi:10.3390/molecules26144288
9. Ming JX, Wang ZC, Huang Y, Ohishi H, Wu RJ, Shao Y, et al. Fucoxanthin extracted from Laminaria Japonica inhibits metastasis and enhances the sensitivity of lung cancer to Gefitinib. J Ethnopharmacol. 2021;265:113302. doi:10.1016/j.jep.2020.113302
10. William Helm C, Christopher States J. Enhancing the efficacy of cisplatin in ovarian cancer treatment - could arsenic have a role. J Ovarian Res. 2009;2:2. doi:10.1186/1757-2215-2-2
11. Materna V, Liedert B, Thomale J, Lage H. Protection of platinum-DNA adduct formation and reversal of cisplatin resistance by anti-MRP2 hammerhead ribozymes in human cancer cells. Int J Cancer. 2005;115(3):393-402. doi:10.1002/ijc.20899
12. Valinezhad Sani F, Palizban A, Mosaffa F, Jamialahmadi K. Glucosamine reverses drug resistance in MRP2 overexpressing ovarian cancer cells. Eur J Pharmacol. 2020;868:172883. doi:10.1016/j.ejphar.2019.172883
13. 13.Kasaian J, Mosaffa F, Behravan J, Masullo M, Piacente S, Iranshahi M. Modulation of multidrug resistance protein 2 efflux in the cisplatin resistance human ovarian carcinoma cells A2780/RCIS by sesquiterpene coumarins. Phytother Res. 2016;30(1):84-9. doi:10.1002/ptr.5504
14. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods. 1983;65:55-63.
15. Valinezhad Sani F, Mosaffa F, Jamialahmadi K, Palizban A. Glucosamine Reverses P-Glycoprotein-Mediated Multidrug Resistance in the Daunorubicin-Resistant Human Gastric Cancer Cells. Nutr Cancer. 2020;72(3):522-7. doi:10.1080/01635581.2019.1636102
16. Jabini R, Ehtesham-Gharaee M, Dalirsani Z, Mosaffa F, Delavarian Z, Behravan J. Evaluation of the cytotoxic activity of crocin and safranal, constituents of saffron, in oral squamous cell carcinoma (KB Cell Line). Nutr Cancer. 2017;69(6):911-19. doi:10.1080/01635581.2017.1339816
17. Momenimovahed Z, Tiznobaik A, Taheri S, Salehiniya H. Ovarian cancer in the world: epidemiology and risk factors. Int J Womens Health. 2019;11:287-99. doi:10.2147/IJWH.S197604.
18. Ye G, Wang L, Yang K, Wang C. Fucoxanthin may inhibit cervical cancer cell proliferation via downregulation of HIST1H3D. J Int Med Res. 2020;48(10):300060520964011. doi:10.1177/0300060520964011.
19. Kim MB, Kang H, Li Y, Park YK, Lee JY. Fucoxanthin inhibits lipopolysaccharide-induced inflammation and oxidative stress by activating nuclear factor E2-related factor 2 via the phosphatidylinositol 3-kinase/AKT pathway in macrophages. Eur J Nutr. 2021;60(6):3315-24. doi:10.1007/s00394-021-02509-z.
20. Zhao Y-Q, Zhang L, Zhao G-X, Chen Y, Sun K-L, Wang B. Fucoxanthin attenuates doxorubicin-induced cardiotoxicity via anti-oxidant and anti-apoptotic mechanisms associated with p38, JNK and p53 pathways. J Funct Foods. 2019;62:103542. doi:10.1016/j.jff.2019.103542.
21. Hudlikar RR, Sargsyan D, Li W, Wu R, Zheng M, Kong AN. Epigenomic, Transcriptomic, and protective effect of carotenoid fucoxanthin in high glucose-induced oxidative stress in mes13 kidney mesangial cells. Chem Res Toxicol. 2021;34(3):713-22. doi:10.1021/acs.chemrestox.0c00235.
22. Zheng J, Tian X, Zhang W, Zheng P, Huang F, Ding G, et al. Protective effects of fucoxanthin against alcoholic liver injury by activation of Nrf2-mediated

antioxidant defense and inhibition of TLR4-mediated inflammation. Mar Drugs. 2019;17(10):552. doi:10.3390/md17100552.
23. Miyashita K, Hosokawa M. Fucoxanthin in the management of obesity and its related disorders. J Funct Foods. 2017;36:195-202. doi:10.1016/j.jff.2017.07.009
24. Eid SY, Althubiti MA, Abdallah ME, Wink M, El-Readi MZ. The carotenoid fucoxanthin can sensitize multidrug resistant cancer cells to doxorubicin via induction of apoptosis, inhibition of multidrug resistance proteins and metabolic enzymes. Phytomedicine. 2020;77:153280. doi:10.1016/j.phymed.2020.153280.
25. Colton SL, Xu XS, Wang YA, Wang G. The involvement of ataxia-telangiectasia mutated protein activation in nucleotide excision repair-facilitated cell survival with cisplatin treatment. J Biol Chem. 2006;281(37):27117-25. doi:10.1074/jbc.M602826200
26. Liu C-L, Chiu Y-T, Hu M-L. Fucoxanthin enhances HO-1 and NQO1 expression in murine hepatic BNL CL. 2 cells through activation of the Nrf2/ARE system partially by its pro-oxidant activity. J Agric Food Chem. 2011;59(20):11344-51. doi:10.1021/jf2029785
27. Kotake-Nara E, Terasaki M, Nagao A. Characterization of apoptosis induced by fucoxanthin in human promyelocytic leukemia cells. Biosci Biotechnol Biochem. 2005;69(1):224-7. doi:10.1271/bbb.69.224
28. Ye G, Lu Q, Zhao W, Du D, Jin L, Liu Y. Fucoxanthin induces apoptosis in human cervical cancer cell line HeLa via PI3K/Akt pathway. Tumour Biol. 2014;35(11):11261-7. doi:10.1007/s13277-014-2337-7
29. Wang L, Zeng Y, Liu Y, Hu X, Li S, Wang Y, et al. Fucoxanthin induces growth arrest and apoptosis in human bladder cancer T24 cells by up-regulation of p21 and down-regulation of mortalin. Acta Biochim Biophys Sin. 2014;46(10):877-84. doi:10.1093/abbs/gmu080
30. Rokkaku T, Kimura R, Ishikawa C, Yasumoto T, Senba M, Kanaya F, et al. Anticancer effects of marine carotenoids, fucoxanthin and its deacetylated product, fucoxanthinol, on osteosarcoma. Int J Oncol. 2013;43(4):1176-86. doi:10.3892/ijo.2013.2019
31. Qu J, Sun Y, Yang L, Niu X, Li L. Fucoxanthin prevents cell growth and induces apoptosis in endometrial cancer HEC-1A cells by the inhibition of the PI3K/Akt/mTOR pathway. J Biochem Mol Toxicol. 2022;36(6):e23027. doi:10.1002/jbt.23027