A COMPREHENSIVE SYSTEMATIC REVIEW EXPLORING FUCOXANTHIN'S POTENTIAL IN CANCER RESEARCH

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Abstract

In traditional medicine, plants have long been seen as strengthening experts. To verify the therapeutic benefits of phytocompounds, several research are being carried out on them. It has been discovered that these substances can fight mild to severe viral and bacterial infections, cancer, and mutagenicity. In addition to being present in various dietary sources, such as vegetables and organic goods, carotenoids are also found in green plants, microbes, and plants. Antioxidants, the capacity to stop dangerous tumor development, and the capacity to induce cancer cell death are only a few of the natural characteristics that carotenoids have in common. Through dietary supplementation, carotenoids can affect gene expression, immunological response, and cell proliferation. Foods cultivated in soil include carotenoids, which humans use as nourishment. Examples include capsanthin, astaxanthin, canthaxanthin, crocetin, lycopene, fucoxanthin, lutein, zeaxanthin, carotene, and cryptoxanthin. Edible brown seaweed includes fucoxanthin. This orange pigment prevents obesity and oxidative stress from causing cancer cells to proliferate. It causes the mitochondrial membrane potential to drop, which sets off cell death. An essential first step in the early stage of the apoptotic pathway via mitochondria is the permeabilization of the mitochondrial membrane. This systematic study explores fucoxanthin's antitumor potential in different cancer types. Using database searches from PubMed, PubMed Central, Google Scholar, Embase, and Web

of Science, we carried out an extensive systematic assessment of publications over the previous 22 years. We were able to obtain 57 relevant papers, including both in-vitro and in-vivo research.

Keywords: Apoptosis, Cancer, Carotenoids, fucoxanthin, phytocomponds

Background

Plant-based herbal medicines have been used in traditional medicine for a very long time. Many investigations are being conducted to examine and validate the therapeutic qualities of different phytocompounds isolated from plants. These phytocompounds include carotenoids, also in fish, vegetables, and microbes. These are lipid-soluble, vividly colored substances (1). Only forty of the 600 carotenoids—categorized as carotenes, xanthophylls, and lycopene—are consumed by humans. Only 20 of them have been found in human tissue and blood samples (2, 3). Some carotenoids, found in many fruits and vegetables, include α-carotene, β-carotene, β-cryptoxanthin, crocetin, lycopene, lutein, fucoxanthin (Fx), capsanthin, astaxanthin (AST), canthaxanthin (CXN), and some others (4,5).

Research demonstrates that when added to the diet, carotenoids have anti-oxidant, anti-tumor, and apoptosis-inducing effects on gene expression, immunity, and cell formation. Breast, cervix, ovary, colon, rectum, and heart cancer rates are all inversely correlated with carotenoid intake. Inflammatory pathways, cell cycle progression, growth factor signaling, and intracellular gap junctional communication all explain this connection (6).

The extract of Paradicsompaprika containing capsanthin (TOMA-P) may have anti-tumor properties against certain cancer types, according to the results of an in-vitro study that tested the extract on three distinct cancer cell lines: Colo201, A549, and HeLa (7). After receiving AST treatment, human colorectal cancer cells (LS-180) underwent apoptosis and eventually died. This was achieved by downregulating the Bcl-2 gene and upregulating the Bax and Caspase-3 genes. Malondialdehyde levels were likewise lowered by increasing the activity of antioxidant enzymes (8). Medical study has demonstrated that astaxanthin enhances tumor immunity in mice by preventing the growth of Meth-A tumor cells. To do this, cytotoxic T lymphocyte activity can be increased, and interferon-γ can be created in response to tumor antigens released by cancerous cells.

Crocetin inhibits DNA synthesis and RNA polymerase II activity by increasing the ratio of Bax to Bcl-2 (10, 11). Crocetin is used to trigger cell death in some different forms of cancer. Through its interference with the Wnt/β catenin pathway, crocin and crocetin have been shown to inhibit the growth of transplantable breast cancer cells (12). Using lycopene resulted in a significant reduction in the severity of pancreatic cancer in men. It prevented the proliferation of oral cancer cells in humans (13, 14). Consuming a wide variety of foods

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that are rich in carotenoid content, such as those that are high in beta-carotene and lycopene, has been shown to have the potential to lower the risk of developing ovarian cancer (15).

Following treatment with Fx, the number of viable cells in the human colon cancer cell lines Caco-2, HT-29, and DLD-1 was shown to be reduced (16). Brain carcinoma is one of the malignancies that exhibits overexpression of PDGF receptors; lutein dramatically reduces PDGF-induced signaling (17).

Fucoxanthin

Its 5,6-monoepoxide structure and allenic bond identify prominent marine carotenoid fucoxanthin (Fx). It is present in seaweeds such as *"Hijikia fusiformis, Undaria pinnatifida, and Sargassum fulvellum,"* it exhibits anti-inflammatory and anticancer effects by reducing free radicals and inducing apoptosis (18). The chemical formula of the carotenoid Fx is C42H58O6. When exposed to light, heat, enzymes, oxygen, unsaturated lipids, and other pro-oxidant chemicals during the extraction, purification, and storage processes, its distinct molecular structure (Figure 1) is insufficient to shield it from natural deterioration. The research highlighted the tight relationship between Fx's absorption, metabolic processes, and bioavailability (19).

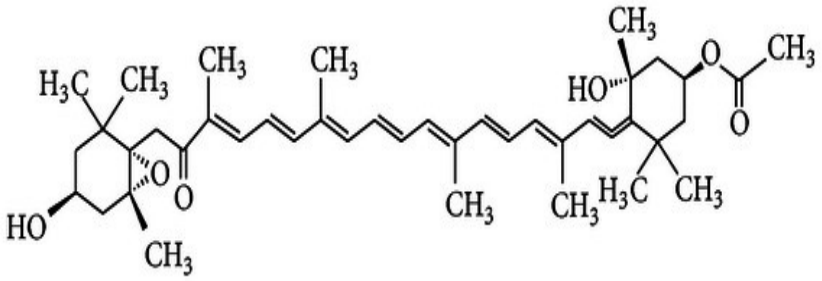


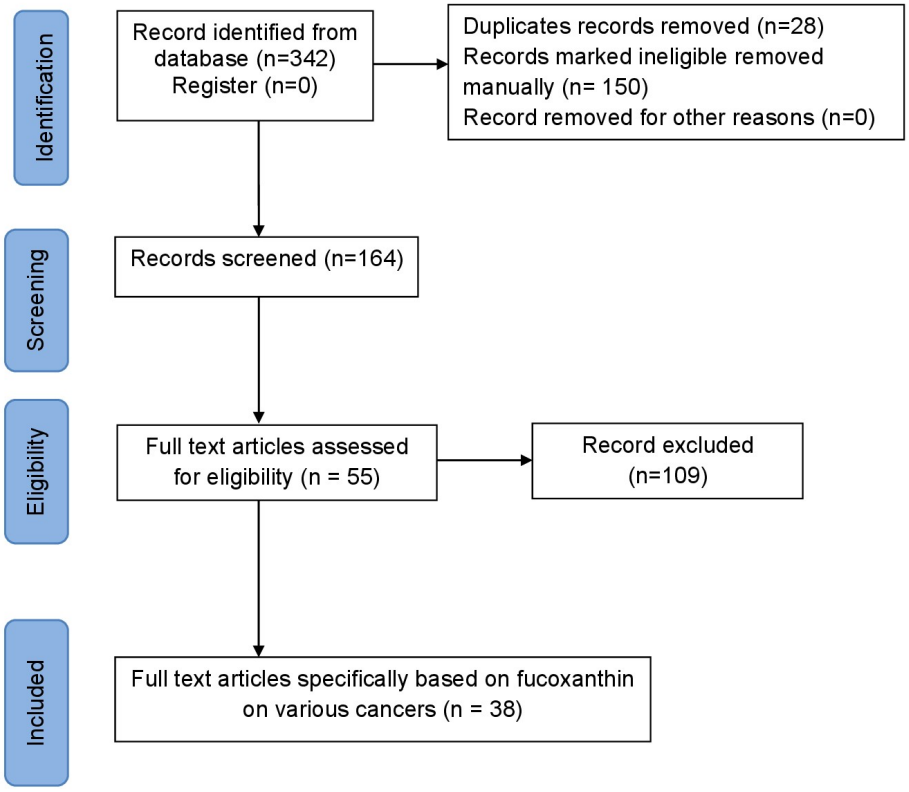
Figure 1: Chemical structure of fucoxanthin (20)

Materials and Methods

The "Recommended Reporting Units for Systematic Reviews" and Meta-analysis (PRISMA) approach (21) was followed in the systematic search, screening, and eventual inclusion of review papers. A comprehensive search was carried out from 2000 to 2022 using a combination of the keywords Fx, capsanthin, astaxanthin (AST), canthaxanthin, crocetin, lycopene, lutein, zeaxanthin, α-carotene, β-carotene, and β-cryptoxanthin in the databases PubMed, PubMed Central, Google Scholar, Embase, and Web of Science. We retrieved all relevant articles that addressed the protective properties of carotenoids against different types of cancer. The suitable selection criteria (the anticancer characteristics of fucoxanthin) were examined in individual records from the first search, and those records were then reexamined using English titles and abstracts. We carefully went over the reference lists of

relevant publications. We referenced the sources to ensure that all pertinent information was included.

Figure 1: **Flowchart of identification of database and search criteria**



Anti-cancer properties of Fucoxanthin on various cancers

Numerous studies have been carried out to examine the effects of Fx on different kinds of cancer tissues.

The effect of Fx on breast cancer

Rwigemera et al. (22) investigated the anticancer effects of Fx and fucoxanthinol (Fxol) on two breast cancer cell lines, MCF-7 and MDA-MB-231, at low doses (10 and 20 µM). By inducing apoptosis, Fx and Fxol at 10 µM demonstrated antiproliferative effects. Fucoxanthinol demonstrated a quicker and more effective inhibition of cell growth in comparison to Fx. Specifically, at a dose of 20 µM, Fxol reduced MCF-7 cell viability by

58% after 48 hours of incubation. However, MDA-MB-231 cell viability decreased to 13% and 47%, respectively, at doses of 10 μM and 20 μM. Moreover, only MDA-MB-231 cells exhibited growth suppression with 10 μM Fxol after 48 hours.

Earlier research has repeatedly shown that Fxol is more potent than Fx. In vitro, fumoxanthinol caused cell death in both estrogen-sensitive MCF-7 and estrogen-resistant MDA-MB-231 breast cancer cell lines. In MDA-MB-231 cells, Fxol attenuated the expression of NF-κB pathway members p65, p50, RelB, and p52 (23). Although women with estrogen receptor-positive cancers often react better to treatment, acquired resistance accounts for around 25% of cases where therapy was not effective. The existence of constitutive NF-κB activity, which promotes estrogen-independent development, maycause this resistance (24, 25).

Significant evidence indicates that essential transcription factors such as asp50, p52, c-Rel, and p100/p52 overexpression are continuously present in the nucleus of tumors that are positive for the estrogen receptor (26). The viewpoint on mammary gland cancer notes increased p65 and p50 dimer production and activation, which increases the transcriptional activity of anti-apoptotic genes (27).

Tumor lymphangiogenesis is a crucial process that shapes endothelial cells into tubular structures within lymphatic vessels and facilitates their infiltration into tumors. Lymphangiogenic factors, such as vascular endothelial growth factor-C (VEFG-C), are secreted by inflammatory and tumor cells, defining this condition. Various concentrations (25, 50, and 100 μMol/l) of Fx were administered to cancer cells for different durations (12, 24, and 48 hours) to assess the impact of Fx. The cells' viability was evaluated using the MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. Furthermore, transwell assays were used to quantify Fx's anti-migration effects on these cells. A matrigel-based experiment was used to investigate the impact of Fx on the development of tube-like structures in lymphatic endothelial cells. Western blot analysis was used to determine the protein expression levels in endothelial cells after they had been treated with Fx for one day. According to the results, Fx successfully blocked the development of tumor lymphangiogenesis, stopping endothelial cells from proliferating, migrating, and forming tube-like structures (28).

By preventing MCF-7 cells from adhering to the endothelium and from migrating through the endothelium, fucoxanthin inhibits CTC-based lung metastasis in vivo. Fx impedes the migration of epithelial cells by blocking the signaling pathways of FAK/Paxillin, PI3K/AKT, and EMT. Based on the results of the hetero-adhesion experiment, carotenoids significantly reduced the formation of CAMs (inflammatory factor-induced cell adhesion molecules) and the adhesion that followed between endothelial cells and MCF-7 cells. The molecular approach involved inhibiting the NF-kB pathway by lowering the phosphorylation levels of IKK-α/H, IkB-α, and NF-kB p65. Fx (25 μM) significantly decreased MCF-7 cell motility

and invasion, according to the findings of the wound-healing and transwell tests. In addition, the ability of cells to migrate across the endothelium was diminished when they were exposed to doses of 5, 10, and 25 μM of fucoxanthin. Fx also significantly decreased the establishment of lung micrometastatic foci in immunologically competent syngeneic mice models of breast cancer metastasis. The antitumor immune responses were enhanced by fucoxanthin, which increased the subsets of cytotoxic T cells in the peripheral immune system. (29)

The effect of Fx on hepatic cell cancer

The Fx shows a potent inhibitory effect on the growth of SK-Hep-1 cells, which are human hepatoma cells, for 24 hours. After 48 hours of exposure, this inhibitory effect was present at Fx doses greater than 1 μM. On the other hand, when it came to BNL CL2 cells (murine embryonic liver cells), Fx first seemed to promote cell development through the 24-hour mark, but, after 48 hours, there was a little decrease in proliferation. These results suggested that processes involving increased intracellular calcium ion (Ca2+) levels and cell gap junction communication were likely responsible for the cell growth arrest and death produced by Fx (30). Furthermore, 48 hours after adding a 10 μM dosage of Fx, a significant 28% decrease in cell growth was seen in human hepatoma HepG2 cells (31).

When HepG2 cells were treated with Fx as opposed to cells treated with a control vehicle, the examination of proteasome activity in these cells showed a significant increase in their function. Subsequent research with Western blot analysis revealed that Fx suppressed cyclin D and that this inhibitory action coincided with the proteasome inhibitor MG132 treatment. Furthermore, Fx was shown to suppress cyclin D's mRNA expression in HepG2 cells by RT-PCR analysis. Encouraging cyclin D's proteasomal breakdown and lowering its transcriptional activity concurrently, fucoxanthin suppresses cyclin D and causes cell cycle arrest. The presence of Fx mostly did not affect the expression of the cyclin-dependent kinase inhibitor p21waf1/Cip1 (32).

The Fx-rich fraction and the crude methanolic extract from Chaetoceroscalcitrans caused apoptosis in the HepG2 cells. Depending on the dosage and length of treatment, the antiproliferative activity against cancer cells was substantially more powerful in the cells treated with the Fx-rich fraction (IC50: 18.89 mg/ml) compared to those treated with the crude methanolic extract (IC50: 87.5 mg/ml) ($p < 0.05$). The cell cycle stopped in the G2/M phase in cells treated with the Fx-rich fraction because of a greater concentration of G0/G1 phase cells in those cells. According to the gene expression analysis, the extract treatment changed the signaling patterns in cancer cells. This included changes to the genes that control oxidative stress (CAT, SOD1, SOD2), cell death (Bax, Bcl-2, BID, CYCS, APAF), and cell signaling (AKT1, ERK1/2, JNK) (33).

Effect of Fx on Prostate Cancer

Fx was discovered to display dose- and time-dependent reduction of cancer cell growth and to stop the cell cycle in the G1 phase when given to prostate cancer cells (DU145). The overexpression of GADD45A was linked to this Fx-induced cell cycle halt. In particular, Fx acted as a cytostatic agent in these cancer cells rather than causing apoptosis (34).

To clarify the roles that GADD45A and MAPK play in relation to the effects of Fx on a prostate cancer cell line that is dependent on androgen consumption, a follow-up experiment was conducted. The results showed that Fx increased the expression of the GADD45A gene, resulting in an arrest in the G1 phase of the cell cycle. The main mechanism that propelled this process from start to finish was the robust activation of the SAPK/JNK signaling pathway. Moreover, Fx showed that it could stop cancer cells from growing, even if there were no visible signs of cell death (35).

There was a decrease in viable cells following treatment with Fx in a different investigation that utilized PC-3 prostate cancer cells. They also found an apoptotic DNA ladder, indicative of preprogrammed cell death. After 72 hours of treatment, Fx at a concentration of 20 μmol/L obtained from U. Pinnatifida significantly decreased the viability of the cells: 5.0% for DU145, 9.8% for lutein CaP, and 14.9% for PC-3. Additional proof of Fx-induced cell death was provided by the TUNEL test, which validated the incidence of DNA fragmentation (36).

Effect of Fx on cancer of the urinary bladder

One of the investigations looked into the possible chemopreventive effects of Fx, an interesting chemical, using trials on EJ-1 bladder cancer cell lines. Using the WST-8 test to determine cell viability, the researchers subjected the cells to several dosages and durations of Fx to assess its influence on cell proliferation. The test was based on formazan formation, correlated with the quantity of viable cells in the culture. They also used Hoechst 33258 stain to look at how the appearance of EJ-1 cells changed. The cells in the control group had uniformly dispersed chromatin and circular nuclei.

On the other hand, apoptotic cells showed nuclear disintegration, tightly packed chromatin, and the distinctive DNA ladder pattern of fragmentation after being exposed to 20 μMFx for 72 hours.

Additionally, the researchers measured the activity caspase-3 at different intervals (12, 24, 48, and 72 hours). One of the enzymes involved in apoptosis is called caspase-3. They discovered that with time, caspase-3 activity dramatically increased. In particular, after 12 hours of incubation, it was 2.6 times greater than the control, peaked at 6.1 times the control level after 24 hours, and then progressively dropped to 3.9 times the control level in the last stages of apoptosis (37).

How Fx suppressed the growth of human T24 bladder cancer cells differed depending on the length of therapy and the amount administered. This inhibition was successfully accomplished with the help of triggering cell cycle arrest in the G0/G1 phase. The hallmarks of this process were a decrease in the expression of cyclin D1, cyclin E, CDK-2, and CDK-4, as well as an increase in p21. This protein inhibits cyclin-dependent kinases (CDKs) (38). Additionally, this phase was characterized by a rise in p21.

Effect of Fx on Colorectal Cancer

Periodically, colorectal cancer stem cells may appear in colon tissue and may function as tumor development indicators. These cells have been shown in xenograft models to display some noteworthy properties, such as drug resistance, multi-potentiality, self-renewal, spherical shape formation, and cancer promotion. Certain surface markers, including CD44, CD166, LGR5, and EpCAM, can be used to detect their presence (39, 40).

In a study using human colorectal cancer cells (HT-29) and CD44high/EpCAM high cells, researchers investigated the antiproliferative effects of Fxol in severe combined immunodeficiency mice. Human cancer prevention is among the many health advantages of Fxol, an intestine metabolite generated by Fx. Significant reduction of CD44high/EpCAMhigh cell proliferation and disruption of tumor cell-produced colon spheres (Csps) were shown by Fxol. Furthermore, in a dosage-dependent way, it caused the cells to generate several condensed chromatin structures. Concerning these modifications in Csps, the IC50 value for Fxol was found to be 1.8 μM. Moreover, PPARβ/δ, PPARγ, and pAkt (Ser473), which are down-regulated by Fxol, are essential for processes such as extracellular adhesion, metastasis, cell proliferation, and the cell cycle. Mice given 5 mg/kg body weight of Fxol for 10 days had significantly fewer Csps tumors than controls. These findings imply that Fxol may provide chemoprevention against human colorectal cancer cells (41).

To investigate any potential anticancer effects using CD-DST (cell viability assay), Fx and Fxol were prescribed to six colorectal cancer cell lines and twenty malignant tissue samples. This was done to determine whether or not there were any anticancer effects. Using the T/C ratio as a marker, the in-vitro sensitivity to Fx and Fxol was assessed. Following exposure to 20 μM doses of Fx and Fxol, three cell lines representing colon cancer—HCT116, Caco 2, and WiDr—showed a significant reduction in T/C ratio, which measures cell viability. It is remarkable that Fxol drastically decreased the T/C ratio in DLD 1 and SSW620 cells, whereas Fx treatment had no impact. Conversely, in Colo205 cells, no carotenoid treatment resulted in a lower T/C ratio.

Furthermore, specimens of malignant tissue treated with 20 and 10 μM doses of Fxol relative to Fx showed a greater T/C ratio (>50%), indicating that Fxol may have a more dramatic cytotoxic impact than Fx on some colorectal tumors. Results drawn from the investigation

revealed that Fx and Fxol may both operate as cytotoxic agents against certain colorectal cancer cell lines and tissue samples (42). These results were based on the effects of both substances on the T/C ratio.

Effect of Fx on human erythrocyte cancer

An independent study sought to demonstrate that Fx causes eryptosis, a condition similar to apoptosis that affects erythrocytes. Before any measurements were made, the erythrocytes in this investigation were incubated for 48 hours in a Ringer solution, with and without different doses of Fx (range from 25 μM to 75 μM). The average forward scatter of erythrocytes was statistically significantly reduced in the presence of Fx at a concentration of 50 μM. Furthermore, after treating erythrocytes with 25 μM Fx for 24 hours, there was a noticeable rise in the proportion of hemolytic erythrocytes, suggesting that Fx treatment impacted hemolysis. Photometry was utilized to ascertain the hemoglobin content in the supernatant to examine the effects of Fx in more detail. According to the study, Fx significantly increased the release of hemoglobin. To calculate cytosolic calcium (Ca++) activity, Fluo3-fluorescence was also quantified as part of the experiment. Fluo3-fluorescence levels rose following a 48-hour exposure to Fx; at 50 μM Fx concentration, statistical significance was obtained. To determine if Fx produced phosphatidylserine translocation or erythrocyte shrinkage, both of which are crucial for the entry of extracellular calcium, erythrocytes were grown for 48 hours under both nominal and real presence of extracellular calcium, as well as 75 μM Fx.

With regard to the scrambling of cell membranes that was brought about by Fx, the findings suggested that the influx of extracellular calcium was partially responsible for producing the phenomenon. The study found that Fx promoted eryptosis, shrinks cells and scrambles cell membranes. This was partly due to higher cytosolic calcium. For example, Fx did not increase oxidative stress (43).

Effect of Fx on cancer of human leukemia cells

The induction of reactive oxygen species (ROS) showed that Fx is responsible for causing HL-60 leukemic cell lines to undergo apoptosis. During the G1 phase of the cell cycle, the cells that were treated with Fx exhibited an increase in the levels of H2O2 and O2¬, in addition to a decrease in the amount of DNA present in the sub-G1 phase. When the cells were subjected to commercial antioxidants such as NAC, there was a reduction in the number of apoptotic bodies and DNA fragmentation that occurred within the cells. As a consequence, the examination revealed that the generation of reactive oxygen species (ROS) was accountable for the cytotoxic impacts of Fx, which in turn led to the death of HL-60 cells (44).

Through the regulation of the production of Bcl proteins (Bcl-xL and Bcl-2) and the cleavage of procaspase-3, -7, and poly (ADP-ribose) polymerase (PARP), it was shown that Fx and Fxol were able to trigger apoptosis in human leukemia cancer cells (HL-60) (45). In contrast, when Fx was given to cells similar to those being investigated, procaspase-3 and PARP cleavage increased. Still, Bax and, Bcl-xL, and Bcl-2 proteins did not (46).

Effect of Fx on cancer of human neural tissues

Fucoxanthin was examined for its ability to shield human neuroblastoma SH-SY5Y cells and primary cerebellar granule neurons (CGNs) against H2O2-induced neurotoxicity. Fucoxanthin prevented H2O2-induced neurotoxicity. Double staining with FDA and PI was utilized to examine viable cells in CGNs to determine the viability of the cells. Surprisingly, the use of 3μMFx significantly decreased the H2O2-induced mortality of neurons. Cell viability was only 48.8% in the H2O2-treated group and rose to 73.4% in the 3 μMFx + H2O2 group. Interestingly, when CGN viability was treated with 3 μMFx alone for 26 hours, no discernible change in viability existed.

Furthermore, when SH-SY5Y cells were subjected to H2O2, there was a notable rise in the number of reactive oxygen species (ROS) found within the cells. The fact that Fx could successfully undo the alterations caused by H2O2 is fascinating. The discovery that Fx is a potential chemical that can reduce the damage that H2O2 causes in brain cells was the result of this discovery. By activating the PI3-K/Akt pathway and blocking the ERK pathway, we accomplished this goal (47). This allowed us to reduce the amount of damage that was done to neuronal cells.

Effect of Fx on skin cancer

An investigation was conducted to find out how Fx may shield human HaCaT keratinocytes from H2O2-induced oxidative damage. During the research, the comet assay was utilized to ascertain the extent of oxidative DNA modification. Fx has been shown to be able to successfully prevent comet tail formation and phospho-histone H2AX synthesis, hence preventing the cellular DNA damage that H2O2 brings about. In keratinocytes, Fx has also demonstrated a protective role by averting oxidative damage and cellular death. By blocking apoptosis-promoting factors such caspase-3, caspase-9, and Bcl-2-associated x protein and simultaneously upregulating the level of an apoptosis inhibitor, Fx reduced cellular death (48).

The possibility of malignant tumors being fatal in the end is primarily reliant on metastasis. In studies, Fx inhibited MMP-9 expression and release, which promotes tumor invasion and migration. Also inhibiting the growth and invasion of highly metastatic melanoma cells was Fx. Additionally, it reduced the expression of cell surface glycoprotein CD44 and CXC chemokine receptor-4 (CXCR4), two proteins implicated in the motility, invasion, and

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adherence of malignant endothelium cells to melanoma cells. These results highlight Fx's anti-metastatic qualities (49).

Effect of Fx on cancerous cells of the cervix

Studies on HeLa cells showed that Fx elicited autophagy, a cellular self-cleaning. Still, it did not cause apoptosis, even in the presence of 3-MA (methyl adenine) pretreatment. This autophagic response was brought about by selectively blocking the Akt/mTOR signaling pathway in cervical cancer cells. As a result, Fx was thought to have the potential to be an anti-carcinogenic agent (50).

In a different study, it was shown that Fx efficiently and concentration-dependently suppressed Akt phosphorylation. Fx also used a caspase-dependent approach to cause cell death in HeLa cell lines. Moreover, Fx had a modest effect on AP-1 activation while decreasing the level of NF-κB activation. The author of the study thus concluded that Fx showed anticancer activity in HeLa cell lines (51).

The HeLa cell PI3K/Akt signaling pathway was examined to discover how Fx affected the situation. After a day of treatment, the effects of different Fx dosages on the proteins involved in the PI3K/Akt signaling pathway were evaluated by Western blot analysis. Furthermore, the western blot findings demonstrated that Fx was responsible for the cleavage of caspase-3, the reduction of Bcl-2 protein levels, the enhancement of Bax synthesis, and the deactivation of the Akt pathway. Based on these findings, it was discovered that Fx inhibited the PI3K/Akt signaling pathway, impeding the growth of HeLa cells. To gain a more in-depth comprehension of the relationship between Fx and nuclear transcription factor activity, the luciferase reporter gene approach was employed to evaluate the activities of NF-ŸB and AP-1. The discovery was made that Fx had a significant influence on AP-1. Still, it had no detectable effect on the level of activation of NF-ΓB proteins. This was confirmed by the findings of a Western blot analysis, which demonstrated that Fx was responsible for increasing the amounts of nuclear NF-ΛB (P65) protein and preventing NF-ΛB from moving from the nucleus to the cytoplasm. These studies' results demonstrated that Fx can potentially cause cell death in HeLa cells via activating NF-κB (52).

Apoptosis that is associated with tumor necrosis factorThis research was conducted on human cervical cancer cell lines SiHa, HeLa, and CaSki. The induced ligand (TRAIL) and Fx mechanism were investigated. In addition to being a member of the Tumor Necrosis Factor (TNF) family, TRAIL can induce apoptosis in cancer cells without compromising their capacity to carry out their normal functions. During the inquiry, flow cytometry was utilized to detect apoptosis, and the XTT method was used to assess the viability of the cells. Specifically, human SiHa cells were treated with Fx at a concentration of 0.5 μMol/L, TRAIL

at a concentration of 100ng/ml, and a combination of Fx and TRAIL at a concentration of 0.5 μMol/L plus 100ng/ml for 48 hours. To determine the levels of protein expression for PI3K/AKT, phosphorylated AKT (P-AKT), PIkBa, and NF-kB (p65), a Western blot experiment was carried out. According to the research findings, cervical cancer cells that were treated with TRAIL and Fx combined displayed a higher level of apoptotic efficiency when compared to cells that were treated with Fx alone or TRAIL alone. According to the study, the PI3K/AKT/NF-κB signaling system can lower the viability of cancer cells and is necessary for the activation of apoptosis by Fx and TRAIL (53).

Our research team evaluated HeLa cells for 24 and 48 hours at different doses (20, 10, 5, 2.5, 1.25, 0.625, 0.3125, and 0.078 μM) to determine the effect of Fx on cell viability. The MTT test measured cell viability, while IC50 values measured Fx's anti-tumor activity. The study's conclusions showed that within the first 24 hours of exposure, Fx showed its strongest anti-cancer effects at a dose of 20 μM. Interestingly, compar the 48 hours, Fx caused a more significant reduction in cell viability at 24 hours. This implies that Fx has a more noticeable impact on cell viability in the shorter time frame. Interestingly, when Fx was compared to tamoxifen, a well-known anti-cancer medication, the effects of Fx were greater at 24 hours than those of tamoxifen at the same time. This finding emphasizes Fx's potential as a worthwhile option for more investigation in treating cervical cancer (54).

Discussion and conclusion

Like Fx, carotenoids have a variety of biological characteristics, including the capacity to cause apoptosis, suppress the growth of malignant tumours, and have antioxidant effects. Although prior research has demonstrated the anti-cancer effects of Fx on various malignancies, relatively few of these studies have a cellular model as their foundation.

Fucoxanthin may stop the P13/Akt pathway, stopping HeLa cell proliferation. It also lowers NF-kappa B's activation level. Numerous malignancies have activated NF-kappa B, which is linked to angiogenesis, proliferation, and inflammation. It promotes the development of tumour cells and mediates the biological signals that suppress apoptosis (52). It is hypothesized that the P13/AKT signalling pathway regulates the cell cycle, apoptosis, and proliferation of cells associated with cervical cancer. Additionally, it is an essential component in the development and progression of a tumour (55). Moreover, Fx scavenges reactive oxygen species (ROS) and cell death to protect keratinocytes from oxidative damage (48).

Fucoxanthin possesses potent antioxidant qualities that can aid in scavenging free radicals, which may contribute to the development of cancer. Cancer risk may be decreased by lowering the pace of cancer cell growth and the level of oxidative stress that these cells encounter. Long-lasting inflammation is a key factor in the development of cancer.

According to studies, fucoxanthin has anti-inflammatory qualities that could help stop the growth of cancer cells and their capacity to spread to other body areas. Fx may be able to assist in lessening the inflammatory milieu that leads to the development of cancer by blocking the pathways that produce inflammatory signals. One natural process that prevents abnormal cells from multiplying is apoptosis. Studies suggest that Fx may trigger apoptosis in some cancer cells. This influence is critical in preventing cancer cells from spreading unchecked. It has been noted that fucoxanthin halts the division and proliferation of cancer cells by inhibiting the cell cycle at certain checkpoints. This particular pathway has potential efficacy in regulating the growth of cancer.

Fucoxanthin has demonstrated encouraging results on a range of cancer cells. It is an interesting molecule because of its anti-proliferative, anti-angiogenic, anti-inflammatory, and apoptosis-inducing qualities. Furthermore, new directions in cancer research and treatment approaches have been made possible by its anti-cancer properties and capacity to amplify the benefits of traditional cancer therapies. Since the majority of studies have been conducted in vitro or using animal models, it is important to emphasize that more clinical research is necessary to validate these findings and establish the appropriate dosage and administration. The study of fucoxanthin is one possible field of research that may be conducted in the ongoing fight against cancer. Fx is a fascinating option for more investigation in cancer research and possible development as an adjuvant therapy or preventive due to its natural origin and varied modes of action. It may also help lower cancer risk when incorporated into a nutritious, well-balanced diet.

References

1. El-Agamey A, Lowe GM, McGarvey DJ, Mortensen A, Phillip DM, Truscott TG et al. (2004). Carotenoid radical chemistry and antioxidant/ pro-oxidant properties. Arch Biochem Biophys 430: 37-48.
2. Rutz, J.K., Borges, C.D., Zambiazi, R.C., da Rosa, C.G. and da Silva, M.M., 2016. Elaboration of microparticles of carotenoids from natural and synthetic sources for applications in food. Food chemistry, 202, pp.324-333.
3. Rao, A.V. and Rao, L.G., 2007. Carotenoids and human health. Pharmacological research, 55(3), pp.207-216
4. Mayne, S.T., 1996. Beta-carotene, carotenoids, and disease prevention in humans. The FASEB Journal, 10(7), pp.690-701.
5. Tanaka, T., Shnimizu, M. and Moriwaki, H., 2012. Cancer chemoprevention by carotenoids. Molecules, 17(3), pp.3202-3242.
6. Milani, A., Basirnejad, M., Shahbazi, S. and Bolhassani, A., 2017. Carotenoids: biochemistry, pharmacology and treatment. British journal of pharmacology, 174(11), pp.1290-1324.

7. Mori, T., Ohnishi M., Komiyama M., Tsutsui A., Yabushita, H and Okada, H., 2002. Growth inhibitory effect of paradicsompaprika in cancer cell lines. Oncology reports, 9(4), pp.807-810.
8. Hormozi, M., Ghoreishi, S. and Baharvand, P., 2019. Astaxanthin induces apoptosis and increases activity of antioxidant enzymes in LS-180 cells. Artificial cells, nanomedicine, and biotechnology, 47(1), pp.891-895.
9. Jyonouchi, H., Sun, S., Iijima, K. and Gross, M.D., 2000. Antitumor activity of astaxanthin and its mode of action. Nutrition and cancer, 36(1), pp.59-65.
10. Bathaie, S.Z., Hoshyar, R., Miri, H. and Sadeghizadeh, M., 2013. Anticancer effects of crocetin in both human adenocarcinoma gastric cancer cells and rat model of gastric cancer. Biochemistry and Cell Biology, 91(6), pp.397-403.
11. Li, S., Jiang, S., Jiang, W., Zhou, Y., Shen, X.Y., Luo, T., Kong, L.P. and Wang, H.Q., 2015. Anticancer effects of crocetin in human esophageal squamous cell carcinoma KYSE-150 cells. Oncology letters, 9(3), pp.1254-1260.
12. Lehmann, B.D., Bauer, J.A., Chen, X., Sanders, M.E., Chakravarthy, A.B., Shyr, Y. and Pietenpol, J.A., 2011. Identification of human triple-negative breast cancer subtypes and preclinical models for selection of targeted therapies. The Journal of clinical investigation, 121(7), pp.2750-2767.
13. Nkondjock, A., Ghadirian, P., Ghadirian, P., Johnson, K, C., Daniel, K.. Canadian Cancer Registries Epidemiology Research Group., 2005. Dietary intake of lycopene is associated with reduced pancreatic cancer risk. The Journal of nutrition, 135 (3), 592-597.
14. Livny, O., Kaplan, I., Reifen, R., Polak-Charcon, S., Madar, Z. and Schwartz, B., 2002. Lycopene inhibits proliferation and enhances gap-junction communication of KB-1 human oral tumor cells. The Journal of Nutrition, 132(12), pp.3754-3759.
15. Cramer, D.W., Kuper, H., Harlow, B.L. and Titus Ernstoff, L., 2001. Carotenoids, antioxidants and ovarian cancer risk in pre and postmenopausal women. International Journal of cancer, 94(1), pp.128-134.
16. Yu, R.X., Hu, X.M., Xu, S.Q., Jiang, Z.J. and Yang, W., 2011. Effects of fucoxanthin on proliferation and apoptosis in human gastric adenocarcinoma MGC-803 cells via JAK/STAT signal pathway. European journal of pharmacology, 657(1-3), pp.10-19.
17. Pietras, K., Sjöblom, T., Rubin, K., Heldin, C.H. and Östman, A., 2003. PDGF receptors as cancer drug targets. Cancer cell, 3(5), pp.439-443.
18. D'Orazio, N., Gemello, E., Gammone, M.A., De Girolamo, M., Ficoneri, C. and Riccioni, G., 2012. Fucoxantin: A treasure from the sea. Marine drugs, 10(3), pp.604-616.
19. Aman, R., Schieber, A. and Carle, R., 2005. Effects of heating and illumination on transcis isomerization and degradation of β-carotene and lutein in isolated spinach chloroplasts. Journal of Agricultural and Food Chemistry, 53(24), pp.9512-9518.

20. Zhang, H., Tang, Y., Zhang, Y., Zhang, S., Qu, J., Wang, X., Kong, R., Han, C. and Liu, Z., 2015. Fucoxanthin: A promising medicinal and nutritional ingredient. Evidence-based complementary and alternative medicine. 723515. doi: 10.1155/2015/723515. Epub 2015 May 27. PMID: 26106437; PMCID: PMC4461761.
21. Page MJ, McKenzie JE, Bossuyt PM, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ. 2021
22. Rwigemera, A., Mamelona, J. and Martin, L.J., 2015. Comparative effects between fucoxanthinol and its precursor fucoxanthin on viability and apoptosis of breast cancer cell lines MCF-7 and MDA-MB-231. Anticancer research, 35(1), pp.207-219.
23. Rwigemera, A., Mamelona, J. and Martin, L.J., 2014. Inhibitory effects of fucoxanthinol on the viability of human breast cancer cell lines MCF-7 and MDA-MB-231 are correlated with modulation of the NF-kappaB pathway. Cell biology and toxicology, 30(3), pp.157-167.
24. Frasor, J., Weaver, A., Pradhan, M., Dai, Y., Miller, L.D., Lin, C.Y. and Stanculescu, A., 2009. Positive cross-talk between estrogen receptor and NF-κB in breast cancer. Cancer research, 69(23), pp.8918-8925.
25. Zubair, A. and Frieri, M., 2013. Role of nuclear factor-κB in breast and colorectal cancer. Current allergy and asthma reports, 13(1), pp.44-49.
26. Cogswell, P.C., Guttridge, D.C., Funkhouser, W.K. and Baldwin, A.S., 2000. Selective activation of NF-κB subunits in human breast cancer: potential roles for NF-κB2/p52 and for Bcl-3. Oncogene, 19(9), pp.1123-1131.
27. Fan, Y., Dutta, J., Gupta, N., Fan, G. and Gélinas, C., 2008. Regulation of programmed cell death by NF-κB and its role in tumorigenesis and therapy. In Programmed cell death in cancer progression and therapy (pp. 223-250). Springer, Dordrecht.
28. Wang, J., Ma, Y., Yang, J., Jin, L., Gao, Z., Xue, L., Hou, L., Sui, L., Liu, J. and Zou, X., 2019. Fucoxanthin inhibits tumour related lymphangiogenesis and growth of breast cancer. Journal of cellular and molecular medicine, 23(3), pp.2219-2229.
29. Wang W, Fu C, Lin M, Lu Y, Lian S, Xie X, Zhou G, Li W, Zhang Y, Jia L, Zhong C, Huang M. 2022. Fucoxanthin prevents breast cancer metastasis by interrupting circulating tumor cells adhesion and transendothelial migration. Front Pharmacol.13: 1-17
30. Liu, C.L., Huang, Y.S., Hosokawa, M., Miyashita, K. and Hu, M.L., 2009. Inhibition of proliferation of a hepatoma cell line by fucoxanthin in relation to cell cycle arrest and enhanced gap junctional intercellular communication. Chemico-biological interactions, 182(2-3), pp.165-172.
31. Liu, C.L., Lim, Y.P. and Hu, M.L., 2013. Fucoxanthin enhances cisplatin-induced cytotoxicity via NFκB-mediated pathway and downregulates DNA repair gene expression in human hepatoma HepG2 cells. Marine drugs, 11(1), pp.50-66.

32. Das, S.K., Hashimoto, T. and Kanazawa, K., 2008. Growth inhibition of human hepatic carcinoma HepG2 cells by fucoxanthin is associated with down-regulation of cyclin D. Biochimica et Biophysica Acta (BBA)-General Subjects, 1780(4), pp.743-749.
33. Foo, S.C., Yusoff, F.M., Imam, M.U., Foo, J.B., Ismail, N., Azmi, N.H., Tor, Y.S., Khong, N.M. and Ismail, M., 2019. Increased fucoxanthin in Chaetoceros calcitrans extract exacerbates apoptosis in liver cancer cells via multiple targeted cellular pathways. Biotechnology Reports, 21, p.e00296.
34. Satomi, Y and Nishino, H., 2007. Fucoxanthin, a natural carotenoid, induces G1 arrest and GADD45 gene expression in human cancer cells. In vivo, 21(2), pp.305-309.
35. Satomi, Y., 2012. Fucoxanthin induces GADD45A expression and G1 arrest with SAPK/JNK activation in lutein Cap human prostate cancer cells. Anticancer research, 32(3), pp.807-813.
36. Kotake-Nara, E., Kushiro, M., Zhang, H., Sugawara, T., Miyashita, K. and Nagao, A., 2001. Carotenoids affect proliferation of human prostate cancer cells. The Journal of nutrition, 131(12), pp.3303-3306.
37. Zhang, Z., Zhang, P., Hamada, M., Takahashi, S., Xing, G., Liu, J. and Sugiura, N., 2008. Potential chemoprevention effect of dietary fucoxanthin on urinary bladder cancer EJ-1 cell line. Oncology reports, 20(5), pp.1099-1103.
38. Wang, L., Zeng, Y., Liu, Y., Hu, X., Li, S., Wang, Y., Li, L., Lei, Z. and Zhang, Z., 2014. 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, 46(10), pp.877-884.
39. Dalerba, P., Dylla, S.J., Park, I.K., Liu, R., Wang, X., Cho, R.W., Hoey, T., Gurney, A., Huang, E.H., Simeone, D.M. and Shelton, A.A., 2007. Phenotypic characterization of human colorectal cancer stem cells. Proceedings of the National Academy of Sciences, 104(24), pp.10158-10163.
40. Vermeulen, L., Todaro, M., de Sousa Mello, F., Sprick, M.R., Kemper, K., Alea, M.P., Richel, D.J., Stassi, G. and Medema, J.P., 2008. Single-cell cloning of colon cancer stem cells reveals a multi-lineage differentiation capacity. Proceedings of the National Academy of Sciences, 105(36), pp.13427-13432.
41. Terasaki, M., Maeda, H., Miyashita, K., Tanaka, T., Miyamoto, S. and Mutoh, M., 2017. A marine bio-functional lipid, fucoxanthinol, attenuates human colorectal cancer stem-like cell tumorigenicity and sphere formation. Journal of Clinical Biochemistry and Nutrition, pp.16-112.
42. Takahashi, K., Hosokawa, M., Kasajima, H., Hatanaka, K., Kudo, K., Shimoyama, N. and Miyashita, K., 2015. Anticancer effects of fucoxanthin and fucoxanthinol on colorectal cancer cell lines and colorectal cancer tissues. Oncology letters, 10(3), pp.1463-1467.

43. Briglia, M., Calabro, S., Signoretto, E., Alzoubi, K., Laufer, S., Faggio, C. and Lang, F., 2015. Fucoxanthin induced suicidal death of human erythrocytes. Cellular Physiology and Biochemistry, 37(6), pp.2464-2475.
44. Kim, K.N., Heo, S.J., Kang, S.M., Ahn, G. and Jeon, Y.J., 2010. Fucoxanthin induces apoptosis in human leukemia HL-60 cells through a ROS-mediated Bcl-xL pathway. Toxicology in vitro, 24(6), pp.1648-1654.
45. Konishi, I., Hosokawa, M., Sashima, T., Kobayashi, H. and Miyashita, K., 2006. Halocynthiaxanthin and fucoxanthinol isolated from Halocynthia roretzi induce apoptosis in human leukemia, breast and colon cancer cells. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, 142(1-2), pp.53-59.
46. Kotake-Nara, E., Asai, A. and Nagao, A., 2005. Neoxanthin and fucoxanthin induce apoptosis in PC-3 human prostate cancer cells. Cancer letters, 220(1), pp.75-84.
47. Yu, J., Lin, J.J., Yu, R., He, S., Wang, Q.W., Cui, W. and Zhang, J.R., 2017. Fucoxanthin prevents H2O2-induced neuronal apoptosis via concurrently activating the PI3-K/Akt cascade and inhibiting the ERK pathway. Food & Nutrition Research, 61(1), p.1304678.
48. Zheng, J., Piao, M.J., Keum, Y.S., Kim, H.S. and Hyun, J.W., 2013. Fucoxanthin protects cultured human keratinocytes against oxidative stress by blocking free radicals and inhibiting apoptosis. Biomolecules & Therapeutics, 21(4), p.270-276
49. Chung, T.W., Choi, H.J., Lee, J.Y., Jeong, H.S., Kim, C.H., Joo, M., Choi, J.Y., Han, C.W., Kim, S.Y., Choi, J.S. and Ha, K.T., 2013. Marine algal fucoxanthin inhibits the metastatic potential of cancer cells. Biochemical and Biophysical Research communications, 439(4), pp.580-585.
50. Hou, L.L., Gao, C., Chen, L., Hu, G.Q. and Xie, S.Q., 2013. Essential role of autophagy in fucoxanthin-induced cytotoxicity to human epithelial cervical cancer HeLa cells. Acta Pharmacologica Sinica, 34(11), pp.1403-1410.
51. Ye, G., Lu, Q., Zhao, W., Du, D., Jin, L. and Liu, Y., 2014. Fucoxanthin induces apoptosis in human cervical cancer cell line HeLa via PI3K/Akt pathway. Tumor Biology, 35(11), pp.11261-11267.
52. Ye, G.L., Jin. L.J., Wang L.L., Dan Du.D.L., 2018. Effect of Fucoxanthin on PI3K/Akt Signaling Pathway in Human Cervical Cancer HeLa Cells. Biomedical Journal of Scientific & Technical Research, 9(3):7218-7223.
53. Jin, Y., Qiu, S., Shao, N. and Zheng, J., 2018. Fucoxanthin and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) synergistically promotes apoptosis of human cervical cancer cells by targeting PI3K/Akt/NF-κB signaling pathway. Medical science monitor: International MedicalJjournal of Experimental and Clinical Research, 24, p.11.
54. Saxena A, Mahajan A, Nagtilak S, Bahuguna SN. In vitro anti-proliferative activity of fucoxanthin on HeLa cells. J Anat Sci. 2020 Dec;28(2): 01-12

55. Shi, X., Wang, J., Lei, Y., Cong, C., Tan, D. and Zhou, X., 2019. Research progress on the PI3K/AKT signaling pathway in gynecological cancer. Molecular Medicine Reports, 19(6), pp.4529-4535.