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Selective Cytotoxic Effect of Astaxanthin on Human Lung and Colon Cancer Cells

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ABSTRACT

Astaxanthin (ASX) is a red xanthophyll carotenoid found in various microorganisms and marine animals. ASX is also called the "super antioxidant" because it has the highest antioxidant activity among existing carotenoids. Studies have shown not only antioxidant properties but also antimicrobial, immunomodulatory, hepatoprotective, anticancer and antidiabetic properties of ASX. However, there is a limited number of studies examining the selective cytotoxic effects of ASX on cancer cells. The aim of this study was to determine the cytotoxic effects of ASX on cells representing common cancer types. For this, human breast (MCF-7), lung (A549), liver (HepG2), melanoma (VMM917), colon (WiDr) cancer and normal fibroblast cells were treated with different concentrations of ASX for 72 h and then the MTT assay protocol was applied. Cisplatin was used as a positive control in cytotoxicity experiments. The results showed that ASX had a dose-dependent cytotoxic effect on all studied cancer cell lines. However, the strongest selective cytotoxic effect of ASX was determined in A549 and WiDr cells compared to fibroblast cells. This study shows that selective cytotoxic effect of ASX should be investigated more extensively, especially in terms of lung and colon cancer.

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Astaksantinin İnsan Akciğer ve Kolon Kanseri Hücrelerindeki Seçici Sitotoksik Etkisi

ÖZET

Astaksantin (ASX), çeşitli mikroorganizmalarda ve deniz hayvanlarında bulunan kırmızı renkli bir ksantofil karotenoididir. ASX, mevcut karotenoidler arasında en yüksek antioksidan aktiviteye sahip olduğundan "süper antioksidan" olarak da adlandırılmaktadır. ASX'in antioksidan özelliğinin yanında, antimikrobiyal, immünomodülatör, hepatoprotektif, antikanser ve antidiyabetik özellikleri de yapılan çalışmalarla gösterilmiştir. Bununla birlikte, ASX'in kanser hücreleri üzerindeki seçici sitotoksik etkilerini inceleyen çalışmalar sınırlıdır. Bu çalışmanın amacı, ASX'in yaygın kanser türlerini temsil eden hücreler üzerindeki sitotoksik etkilerini belirlemektir. Bunun için insan meme (MCF-7), akciğer (A549), karaciğer (HepG2), melanoma (VMM917), kolon (WiDr) kanseri ve normal fibroblast hücreleri 72 saat boyunca farklı konsantrasyonlarda ASX ile muamele edildi ve ardından MTT protokolü uygulandı. Cisplatin sitotoksisite deneylerinde pozitif kontrol olarak kullanıldı. Sonuçlar, ASX'in incelenen tüm kanser hücre hatları üzerinde doza bağımlı bir sitotoksik etkiye sahip olduğunu gösterdi. Bununla birlikte, fibroblast hücreleri ile kıyaslandığından ASX'in en güçlü seçici sitotoksik etkisinin A549 ve WiDr hücrelerinde olduğu belirlendi. Bu çalışma ASX'in seçici sitotoksik etkisinin, özellikle akciğer ve kolon kanseri açısından daha kapsamlı şekilde araştırılması gerektiğini göstermektedir.

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INTRODUCTION

Cancer is a multifactorial heterogeneous disease and cancer cells are characterized by basic distinctive abilities, such as uncontrolled cell proliferation, avoiding apoptosis, invasion, metastasis and angiogenesis (Kavitha et al., 2013). Proto-oncogenes becoming oncogenes and inactivation of tumor suppressor genes are the basic molecular mechanisms that cause the development of cancer (Kowshik et al., 2019). Today various methods, such as chemotherapeutic drugs, radiotherapy, immunotherapy and hormone therapy are used alone and/or with combination in cancer treatment (Shanmugapriya et al., 2019). The chemotherapy method, which uses traditional anticancer drugs in both clinical applications and cancer studies, has proven to be effective in the treatment of tumors (Chen et al., 2017). Although chemotherapy is frequently used in cancer therapies, some disadvantages, such as drug resistance, side effects, and nonspecific cytotoxic cellular damage reduce the its percentage of success (Nagendraprabhu and Sudhandiran, 2011; Chen et al., 2017). Various approaches are suggested to overcome these disadvantages of chemotherapy. One of these approaches is complementary and alternative medicine (CAM), which involves the use of compounds derived from natural products with high anticancer efficacy but low side effects (Chen et al., 2017). The demonstration of the ability of antioxidant phytochemicals to suppress intracellular signaling cascade has led them to be considered as a new generation anticancer agent in recent years (Kavitha et al., 2013). Researches in the field of cancer, focusing on energy metabolism and oxidative stress, draw attention to the anticancer properties of antioxidants. Emerging data show that antioxidant agents play an important role in cancer treatment with their pro-oxidant character (Li et al., 2015). Therefore, it is a popular area of research to investigate the possibility that compounds with strong antioxidant activity may be new and effective agents for cancer treatment (Yan et al., 2017).

Carotenoids are synthesized *de novo* by some microorganisms, plants and algae. In nature, there are more than 700 carotenoids and which are classified as carotenes and xanthophylls (Vijay et al., 2016). Astaxanthin (ASX), a xanthophyll carotenoid, is chemically defined as 3,3'-dihydroxy-β,β'-carotene-4,4'-dion (Ekpe et al., 2018). ASX is the main carotenoid pigment found in aquatic animals and is found in most of the favorite seafood, such as salmon, trout, red sea bream, shrimp, lobster and fish roe. It is also found in some birds, such as flamingo and quail. ASX is involved in processes, such as protecting biomolecules against oxidation and UV light, regulating the immune response, pigmentation, communication and reproductive behavior in naturally produced organisms (Guerin et al., 2003). It is reported that the activity of ASX to scavenge the reactive oxygen species is 10 times more than zeaxanthin, lutein, and canthaxanthin and 100 times more than α-tocopherol (Ambati et al., 2014). Thus, ASX is known as the "king of carotenoids" due to its strong antioxidant properties (Hormozi et al., 2019). The super antioxidant property of ASX is attributed to the presence of hydroxyl and keto groups on each ionone ring in its structure (Guerin et al., 2003). It was firstly approved by the U.S. Food and Drug Administration (USFDA) as a feed additive for use in the aquaculture industry in 1987 and was subsequently approved by same council for use as a dietary supplement in 1999 (Zhang and Wang, 2015). ASX cannot be synthesized by animals and humans, it should be taken with a diet (Guerin et al., 2003). The use of ASX as a nutritional supplement in food, feed, nutraceuticals and pharmaceuticals is therefore growing rapidly (Ambati et al., 2014). Products containing ASX are becoming increasingly popular, with a market size of over US$100 million in 2018 and double-digit annual growth rates (Brendler and Williamson, 2019). The fact that no side effects were reported even at high concentrations of ASX increases the interest in it day by day (Zhang and Wang, 2015; Hormozi et al., 2019). It has many beneficial biological activities, such as antioxidant, antidiabetic, anti-inflammatory, neuroprotective, hepatoprotective, skin protective, anti-ulcerative, immunomodulator, cardioprotective, and anticancer (Ambati et al., 2014; Ekpe et al., 2018; Fakhri et al., 2018). ASX is therefore seen as an important potential treatment tool for various diseases such as inflammatory, metabolic, neurodegenerative and cancer. Among these diseases, various studies have been carried out in recent years about ASX, especially in the field of cancer (Shao et al., 2016). It is reported that ASX exhibits anticancer and antimetastatic activity in many types of cancer, such as bladder (Tanaka et al., 1994), breast (Nakao et al., 2010), colon (Nagendraprabhu and Sudhandiran, 2011) and oral (Kavitha et al., 2013) carcinogenesis. However, there is no study investigating the selective cytotoxic effects of ASX on common cancer types. We therefore aimed to investigate the cytotoxic effect of ASX on five common cancer (melanoma, lung, breast, liver and colon) and one normal cell lines in this study.

MATERIALS and METHOD

Chemicals

Astaxanthin was purchased from Sigma-Aldrich (St. Louis, MO, USA) and dissolved in dimethyl sulfoxide (DMSO) solution. All other chemical and solutions used cytotoxicity experiments were purchased from Sigma-Aldrich (St. Louis, MO, USA), Lonza (Verviers, Belgium) and Biological Industries (Kibbutz Beit Haemek, Israel).

Cell Culture

Human melanoma (VMM917), breast (MCF-7), colon (WiDr), lung (A549), liver (HepG2) cancer and normal fibroblast cells were purchased from American Type Culture Collection (Manassas, VA, USA). All cell lines were maintained in Eagle's minimum essential medium (EMEM) supplemented with 10% fetal bovine serum, 1% penicillin/streptomycin solution and with a 5% CO_2 supply at 37°C (Turan et al., 2018; Demir et al., 2018a).

3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyl-Tetrazolium Bromide (MTT) Assay

Cytotoxic effect of ASX was examined on cancer and normal cells using MTT assay with slight modification (Mosmann, 1983; Demir et al., 2018b). Briefly, all cells were inoculated in 96-well plates at a density of 5×10^3 cells/well overnight prior to ASX and cisplatin (was used as a positive control) treatment. Fibroblast cells were inoculated in 96-well plates at a density of 2.5×10^3 cells/well (Demir et al., 2019a; Demir et al., 2019b). Then, after adding the agents in various concentrations (ASX: 1.25-250 µM; cisplatin: 0.3125-40 µM) incubation was performed at 37°C for 72 h (Li et al., 2015; Shao et al., 2016; Turan et al., 2017; Demir et al., 2018b). After the incubation, 10 µL of MTT solution dissolved in phosphate buffer saline were added to each well (0.25 mg mL^{-1}) and further incubated at 37°C for 2 h. Then, the media were discarded and added 200 µL of DMSO in each well. The optical density (OD) of the purple color formed was measured using a microplate reader (Versamax Molecular Devices, CA, USA) at 570 nm (Aliyazicioglu et al., 2019). Cell viability (%) was calculated used with following formula (Shanmugapriya et al., 2019):

$$\text{Cell viability (\%)} = (OD_{\text{treatment group}} / OD_{\text{control group}}) \times 100$$

Dose-response curves were drawn using %logarithmic concentrations against cell viability and the IC_{50} value of ASX and cisplatin were calculated for each cell line (Demir et al., 2020). IC_{50} values calculated for ASX and cisplatin were used to determine the selectivity index (SI) value with the following formula (Turan et al., 2019):

$$SI = \text{Fibroblast cells } IC_{50} / \text{Cancer cells } IC_{50}$$

Statistical Analysis

All cytotoxicity experiments were performed four times. The distribution of the data was examined with the Kolmogorov-Smirnov test. Data showing normal distribution were expressed as arithmetic mean±standard deviation. Statistical analyzes between the groups were revealed by ANOVA and post-hoc Tukey tests. $p<0.01$ was regarded as significant.

RESULTS and DISCUSSION

The World Health Organization defines CAM as a large group of healthcare services implemented in a country before being integrated into the traditional healthcare system and traditional medical practices (Chen et al., 2017). The use of natural product-derived compounds or extracts in cancer prevention and cancer treatment is increasingly accepted in the scientific community due to its cheap costs, easy accessibility and low toxicity (Kim et al., 2016). Increasing evidence suggests that natural agents destroy cancer development by preventing the onset of carcinogenesis, stopping tumor progression, or killing cancer cells. Studies in this area have become very popular in recent years and the anticancer activity of many natural compounds has been demonstrated by experimental studies (Jyonouchi et al., 2000). Dietary carotenoids have gained nutritional importance due to their important role in reducing cardiovascular disease, cancer, obesity and age-related degenerative diseases (Vijay et al., 2016). ASX is a ketocarotenoid red pigment commonly found in nature, especially in microalgae, red yeast and some marine animals, such as salmon, shrimp, lobster and crayfish, and has higher antioxidant activity than other carotenoids, vitamins and phytochemicals (Kavitha et al., 2013; Vijay et al., 2016). ASX is therefore seen as an important potential treatment tool for various chronic diseases, including cancer (Shao et al., 2016). Increasing evidence suggests that ASX may be a new and promising chemotherapeutic agent to inhibit proliferation of various cancer cells (Ambati et al., 2014). However, studies investigating the cytotoxic effects of ASX on common cancer types are limited (Tanaka et al., 1994; Nakao et al., 2010; Nagendraprabhu and Sudhandiran, 2011; Kavitha et al., 2013). Therefore, the cytotoxic effects of ASX on lung, liver, colon, breast and melanoma cancer cells, which are the common types of cancer in the world, were therefore determined using MTT assay and the growth curves of the cells were shown in Figure 1. Although, the growth curves showed that ASX exhibits cytotoxic effect in all studied cancer cells in a dose-dependent manner, the strongest cytotoxic effect was determined in A549 and WiDr cells.

IC_{50} values obtained as a result of analysis of growth curves were presented in Table 1. The data in Table 1 clearly show that the cell lines where ASX is most effective are A549 and WiDr.

Selectivity is a parameter that reveals the level of cytotoxic effect of a molecule on cancer cells compared to normal cells, and is one of the most important criteria for a compound to be evaluated as a chemotherapeutic (Demir et al., 2019b). For this reason, one normal fibroblast cell line was used along with five cancer cells in the study.

The SI of the ASX and cispatin for all studied cancer cells were calculated using the formula described in the "Materials and Method Section" of the IC_{50} values obtained for each cell and results were presented in Table 2.

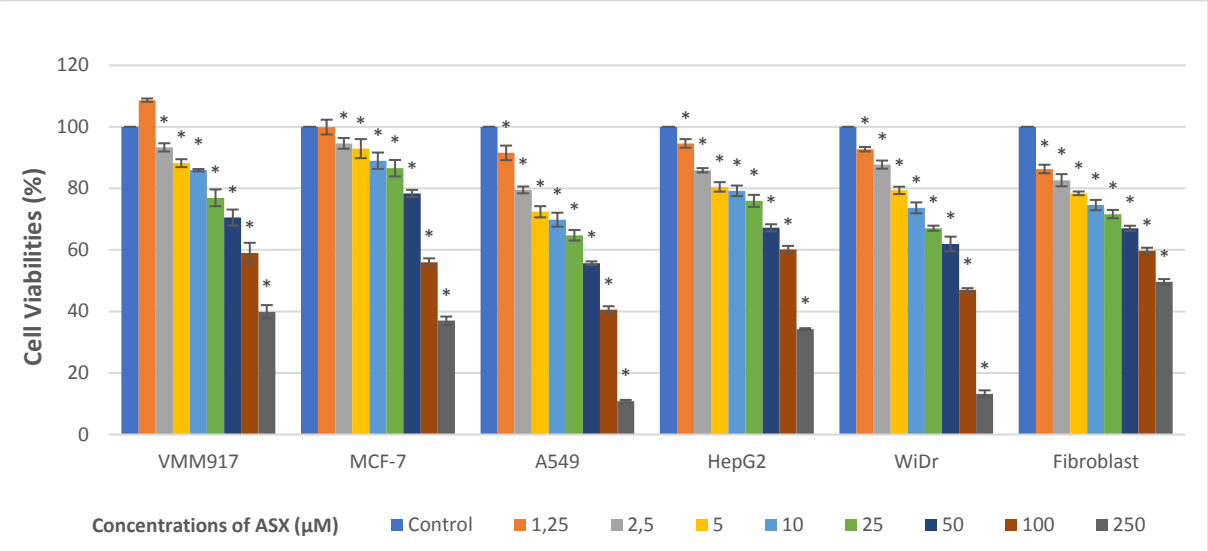

Figure 1. The cytotoxic effect of ASX on cancer and fibroblast cell lines. *Denotes statically significant differences in comparison with control ($p<0.01$).

*Şekil 1. ASX'in kanser ve fibroblast hücre hatlarındaki sitotoksik etkisi. *Kontrol ile kıyaslandığında istatistiksel olarak anlamlı farkı belirtir ($p<0.01$).*

Table 1. IC_{50} values (µM) calculated for ASX and cisplatin (n=4)

Çizelge 1. ASX ve cisplatin için hesaplanan IC_{50} değerleri (µM) (n=4)

Cell Line	ASX	Cisplatin
VMM917	156.1±9.1	2.32±0.03
MCF-7	158.5±2.7	3.63±0.21
A549	61.9±1.7	2.08±0.06
HepG2	143.2±3.7	9.21±0.10
WiDr	75.1±3.1	2.76±0.22
Fibroblast	209.1±4.4	12.22±0.17

Table 2. SI values of ASX and cisplatin

Çizelge 2. ASX ve cisplatinin SI değerleri

Cell Lines	Test Compounds	
	ASX	**Cisplatin**
VMM917	1.34	5.27
MCF-7	1.32	3.37
A549	3.38	5.88
HepG2	1.46	1.33
WiDr	2.78	4.43

The data in Table 2 showed that ASX exhibits a highly selective cytotoxic effect, especially in the A549 and WiDr cell lines.

Lung cancer is one of the deadliest malignancies in the world, and non-small cell lung cancer (NSCLC) accounts for more than 85% of all lung cancer cases (Liao et al., 2016). Colon cancer is the third most malignant neoplasm in the world and remains an important cause of mortality in Asian and Western countries (Nagendraprabhu and Sudhandiran, 2011). Traditional medical interventions such as surgical resection, radiotherapy, chemotherapy and immunotherapy are generally used in the treatment of colon and lung cancer. Although chemotherapy is frequently used in the treatment of these cancers, some disadvantages, such as drug resistance and cytotoxicity in normal cells, of chemotherapy reduce the percentage of success. Scientists have therefore, turned to alternative or complementary strategies. In this sense, natural product-based compounds that have been used in traditional therapy for centuries are frequently researched for their anticancer activities (Nagendraprabhu and Sudhandiran, 2011; Liao et al., 2016). From this point of view, we think that the results of this study, which examined the selective cytotoxic effect of ASX on A549 and WiDr cells for the first time, are important.

In previous studies investigating the cytotoxic effect of ASX on various cancer cells, Anderson (2005) demonstrated that ASX decreases the growth of prostate cancer (LNCaP) cells through inhibited the activity of 5α-reductase, while Lim *et al.* (2011) reported that ASX inhibits the proliferation of esophageal cancer (TE-4) cells in a dose-dependent manner via induced caspase-dependent apoptosis and the cell cycle arrest. Li *et al.* (2015) demonstrated that ASX inhibits the proliferation of liver cancer (LM3 and SMMC-7721) cells in a concentration-dependent manner via enhanced apoptosis and inhibited nuclear factor kappa B (NF-κB) and Wnt/B-catenin signaling pathways, while Liu *et al.* (2016) reported that ASX exhibits antiproliferative effect on colon cancer (HCT116 and HT29) cells in a concentration-dependent manner through inducing cell cycle arrest at G_2/M phase and apoptosis. Kim *et al.* (2016) demonstrated that ASX exhibits antiproliferative effect on various gastric adenocarcinoma (KATO-III, MKN-45 and SNU-1) cells

through arrested the cycle at G_1 phase, while Vijay *et al.* (2016) reported that ASX exhibits antiproliferative effect on human leukemia (HL-60) cells through inducing oxidative stress. Chen *et al.* (2017) demonstrated that ASX inhibits the growth and metastasis of human melanoma (A2058 and A375) cells through inducing apoptosis and cell cycle arrest, while Kowshik *et al.* (2019) reported that ASX shows cytotoxic effect on human oral squamous cell carcinoma (SCC131 and SCC4) cells through inducing ROS-independent mitochondrial apoptosis pathway.

Previous reviews have been suggesting that ASX can exhibit anticancer effects by regulating the nuclear factor erythroid 2-related factor 2 (Nrf2) pathway, inhibiting cell proliferation, inducing apoptosis, and modulating the immune response, oxidative stress and various cellular signaling pathways, such as NF-κB, Wnt/ß-catenin, MAPK/ERK kinase kinase (MEKK) and phosphoinositide 3-kinases/protein kinase B (PI3K/Akt) (Kavitha et al., 2013; Zhang and Wang, 2015; Kowshik et al, 2019). We are speculated that the selective cytotoxic effect of ASX especially on lung and colon cancer cell lines is due to its ability of modulating these signaling pathways.

CONCLUSION

This study is the first to reveal the selective cytotoxic effect of ASX in colon and lung cancer cells. However, more detailed studies including interrelated molecular pathways can provide more evidence.

Researchers Contribution Rate Declaration Summary

The authors declare that they have contributed equally to the article.

Conflicts of Interest Statement

None of the authors had any financial or personal relationships with other individuals or organizations that might inappropriately influence their work during the submission process.

REFERENCES

Aliyazicioglu Y, Demir S, Yaman SO, Sener SO, Demir EA, Aliyazicioglu R, Turan I 2019. Phytochemical analysis of *Dorycnium pentaphyllum* and its antiproliferative effect on cervix cancer cells. *KSU J Agric Nat*, 22(Suppl 2): 365-373.

Ambati RR, Phang SM, Ravi S, Aswathanarayana RG 2014. Astaxanthin: Sources, extraction, stability, biological activities and its commercial applications-A review. *Mar Drugs*, 12: 128-152.

Anderson ML 2005. A preliminary investigation of the enzymatic inhibition of 5α-reductase and growth of prostatic carcinoma cell line LNCap-FGC by natural astaxanthin and saw palmetto lipid extract *in vitro*. *J Herb Pharmacother*, 5(1): 17-26.

Brendler T, Williamson EM 2019. Astaxanthin: How much is too much? A safety reviews. *Phytother Res*, 33: 3090-3111.

Chen YT, Kao CJ, Huang HY, Huang SY, Chen CY, Lin YS, Wenf ZH, Wang HMD 2017. Astaxanthin reduces MMP expressions, suppresses cancer cell migrations, and triggers apoptotic caspases of *in vitro* and *in vivo* models in melanoma. *J Funct Foods*, 31: 20-31.

Demir S, Turan I, Aliyazicioglu Y 2018a. Cytotoxic effect of *Rhododendron luteum* leaf extract on human cancer cell lines. *KSU J Agric Nat,* 21(6): 950-956.

Demir S, Turan I, Aliyazicioglu R, Ozer Yaman S, Aliyazicioglu Y 2018b. *Primula vulgaris* extract induces cell cycle arrest and apoptosis in human cervix cancer cells. *J Pharm Anal*, 8(5): 307-311.

Demir S, Turan I, Aliyazicioglu Y 2019a. Antioxidant properties of *Primula vulgaris* flower extract and its cytotoxic effect on human cancer cell lines. *KSU J Agric Nat*, 22(1): 78-84.

Demir S, Turan I, Misir S, Aliyazicioglu Y 2019b. Selective cytotoxic effect of *Dorycnium pentaphyllum* extract on human breast, liver, and lung cancer cells. *KSU J Agric Nat*, 22(3): 473-479.

Demir S, Ozer Yaman S, Sener SO, Ayazoglu Demir E, Aliyazicioglu R, Ozgen U, Mentese A, Deger O, Aliyazicioglu Y 2020. *Dorycnium pentaphyllum* extract has antiproliferative effect on human cervix and colon cancer cells. *Nutr Cancer*, 72(3): 504-512.

Ekpe L, Inaku K, Ekpe V 2018. Antioxidant effects of astaxanthin in various diseases-a review. *J Mol Pathophysiol*, 7(1): 1-6.

Fakhri S, Abbaszadeh F, Dargahi L, Jorjani M 2018. Astaxanthin: A mechanistic review on its biological activities and health benefits. *Pharmacol Res*, 136: 1-20.

Guerin M, Huntley ME, Olaizola M 2003. *Haematococcus* astaxanthin: applications for human health and nutrition. *Trends Biotechnol*, 21(5): 210-216.

Hormozi M, Ghoreishi S, Baharvand P 2019. Astaxanthin induces apoptosis and increases activity of antioxidant enzymes in LS-180 cells. *Artif Cells Nanomed Biotechnol*, 47(1): 891-895.

Jyonouchi H, Sun S, Iijima K, Gross MD 2000. Antitumor activity of astaxanthin and its mode of action. *Nutr Cancer*, 36(1): 59-65.

Kavitha K, Kowshik J, Kishore TK, Baba AB, Nagini S 2013. Astaxanthin inhibits NF-κB and Wnt/ß-catenin signaling pathways via inactivation of Erk/MAPK and PI3K/Akt to induce intrinsic apoptosis in a hamster model of oral cancer. *Biochim Biophys Acta*, 1830(10): 4433-4444.

Kim JH, Park JJ, Lee BJ, Joo MK, Chun HJ, Lee SW, Bak YT 2016. Astaxanthin inhibits proliferation of human gastric cancer cell lines by interrupting cell cycle progression. *Gut and Liver*, 10(3): 369-374.

Kowshik J, Nivetha R, Ranjani S, Venkatesan P, Selvamuthukumar S, Veeravarmal V, Nagini S 2019. Astaxanthin inhibits hallmarks of cancer by targeting the PI3K/NF-κB/STAT3 signalling axis in oral squamous cell carcinoma models. *IUBMB Life*, 71(10): 1595-1610.

Li J, Dai W, Xia Y, Chen K, Li S, Liu T, Zhang R, Wang J, Lu W, Zhou Y, Yin Q, Abudumijiti H, Chen R, Zheng Y, Wang F, Lu J, Zhou Y, Guo C 2015. Astaxanthin inhibits proliferation and induces apoptosis of human hepatocellular carcinoma cells via inhibition of NF-KB, p65 and Wnt/B-catenin *in vitro*. *Mar Drugs*, 13: 6064-6081.

Liao KS, Wei CL, Chen JC, Zheng HY, Chen WC, Wu CH, Wang TJ, Peng YS, Chang PY, Lin YW 2016. Astaxanthin enhances pemetrexed-induced cytotoxicity by downregulation of thymidylate synthase expression in human lung cancer cells. *Regul Toxicol Pharmacol*, 81: 353-361.

Lim S, Lee JY, Jung WH, Lim EH, Joo MK, Lee BJ, Park JJ, Kim JS, Bak YT, Jung SW, Lee SW 2011. Anticancer effects of astaxanthin and α-tocopherol in esophageal cancer cell lines. *Korean J Helicobacter Up Gastrointest Res*, 11(3): 170-175.

Liu X, Song M, Gao Z, Cai X, Dixon W, Chen X, Cao Y, Xiao H 2016. Stereoisomers of astaxanthin inhibit human colon cancer cell growth by inducing G_2/M cell cycle arrest and apoptosis. *J Agric Food Chem*, 64(41): 7750-7759.

Mosmann T 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J Immunol Methods*, 65: 55-63.

Nagendraprabhu P, Sudhandiran G 2011. Astaxanthin inhibits tumor invasion by decreasing extracellular matrix production and induces apoptosis in experimental rat colon carcinogenesis by modulating the expressions of ERK-2, NFkB and COX-2. *Invest New Drugs*, 29: 207-224.

Nakao R, Nelson OL, Park JS, Mathison BD, Thompson PA, Chew BP 2010. Effect of dietary astaxanthin at different stages of mammary tumor initiation in BALB/c mice. *Anticancer Res*, 30(6): 2171-2175.

Shanmugapriya K, Kim H, Kang HW 2019. In vitro antitumor potential of astaxanthin nanoemulsion against cancer cells via mitochondrial mediated apoptosis. *Int J Pharm*, 560: 334-346.

Shao Y, Ni Y, Yang J, Lin X, Li J, Zhang L 2016. Astaxanthin inhibits proliferation and induces apoptosis and cell cycle arrest of mice H22 hepatoma cells. *Med Sci Monit*, 22: 2152-2160.

Tanaka T, Morishita Y, Suzui M, Kojima T, Okumura A, Mori H 1994. Chemoprevention of mouse urinary bladder carcinogenesis by the naturally occurring carotenoid astaxanthin. *Carcinogenesis*, 15(1): 15-19.

Turan I, Demir S, Kilinc K, Aliyazicioglu Y, Alver A, Misir S, Ozer Yaman S, Akbulut K, Mentese A, Deger O 2017. *Morus rubra* extract induces G1 cell cycle arrest and apoptosis in human lung and prostate cancer cells. *IJPER*, 51(1): 51-58.

Turan I, Demir S, Kilinc K, Yaman SO, Misir S, Kara H, Genc B, Mentese A, Aliyazicioglu Y, Deger O 2018. Cytotoxic effect of *Rosa canina* extract on human colon cancer cells through repression of telomerase expression. *J Pharm Anal*, 8(6): 394-399.

Turan I, Demir S, Aliyazicioglu R, Kilinc K, Ozer Yaman S, Akbulut Cakiroglu K, Kanbolat S, Ayazoglu Demir E, Mentese A, Aliyazicioglu Y, Deger O 2019. Dimethyl sulfoxide extract of *Dianthus carmelitarum* induces S phase arrest and apoptosis in human colon cancer cells. *Nutr Cancer*, 71(7): 1181-1188.

Vijay K, Sowmya PR, Arathi BP, Lakshminarayana R 2016. Evaluation of anti-inflammatory and anti-proliferative effect of hydroxy-, keto-, and epoxy-carotenoids in RAW 264.7 and HL-60 cells. *J Food Chem Nanotechnol*, 2(3): 153-161.

Yan T, Li HY, Wu JS, Niu Q, Duan WH, Han QZ, Ji WM, Zhang T, Lv W 2017. Astaxanthin inhibits gemcitabine-resistant human pancreatic cancer progression through EMT inhibition and gemcitabine resensitization. *Oncol Lett*, 14(5): 5400-5408.

Zhang L, Wang H 2015. Multiple mechanisms of anti-cancer effects exerted by astaxanthin. *Mar Drugs*, 13: 4310-4330.