REVIEW ARTICLE

In Vitro and in Vivo Cytotoxic Effects of Chlorella Against Various types of Cancer

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ABSTRACT

Chlorella is one of the microalgae that had been studied intensively owing to its rapid growth and easily cultured at a large scale compared to other microalgae and valuable nutrient compositions. Numerous studies have observed that Chlorella possess various health benefit including antioxidant, anti-cholesterol, anti-inflammatory, and antiproliferative effect against many types of cancer. In this review paper, the effects of various Chlorella species against cancer cells and animal induced cancer are discussed and an overview on Chlorella is briefed. The Chlorella deleterious effect on cancer through various mechanisms such as enhancement of immune system and apoptosis; improving lipid peroxidation; synthesis and expression of the protein-degrading matrix; and preventing the formation of new blood vessels are elaborated as well. Based on the findings of many studies reported in this article, it can be suggested that Chlorella has the potential in supporting cancer therapy and may develop to become an anti-cancer agent.

KEYWORDS: Chlorella, cancer, anti-proliferative, mechanism

INTRODUCTION

Cancer is the leading cause of death in the world with an estimated 18.1 million new cancer cases and 9.6 million cancer deaths in 2018 despite existence.[1] There has been a steadfast rise in cancer incidence and mortality globally every year due to an increase in population and age. According to the report of the International Agency for Research on Cancer[2], nearly half of the diagnosed cancer cases and more than half of the cancer deaths worldwide occur in Asia partly due to 60% of the global population comes from this region. Europe and the Americas recorded 23.4% and 21.0% of the global cancer cases, respectively, and 20.3% and 14.4% of the cancer death, respectively.

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Currently, cancer is treated by surgery, radiation therapy, and chemotherapy in which surgery involves tumour removal; radiation therapy includes exposure of the tumour to radiation and chemotherapy involves drug administration into the patient's body.[3] Often, radiation therapy, and chemotherapy are used alone or in combination, before or after surgery to destroy tumour remnants and reduce the cancer recurrence. However, chemotherapy proved to be the most effective treatment as the drug travels throughout the entire body to kill any cancer cells. Unfortunately, both chemotherapy and radiotherapy always comes with toxicity either to normal cells neighbouring the cancer bulk[4] or the distance sites in various organs and cause hepatotoxicity, cutaneous toxicity, cardiotoxicity, and abnormal bone and mineral metabolism.[5] High-dose chemotherapy often led to ROS-induced cytotoxicity and kills normal cells. Thus, a combination of chemotherapy with antioxidants may improve chemotherapy treatment. One study showed the extract of microalgae particularly Chlorella extract not only has

an anti-cancer effect against hepatocellular cancer cell line (HepG2) and Ehrlich ascites carcinoma cell, it also exhibits antioxidant activity.[6] Administration of Chlorella glycoprotein was also observed to reduce the side effect of 5-fluorouracil (5 FU), a chemotherapy drug that causes myelosuppression by accelerating the recovery of hematopoietic stem cells.[7] It was found the Chlorella glycoprotein exerted this positive effect without affecting the antitumor activity of the drug. These attributes made Chlorella attractive to be used in combination or adjuvant with standard chemotherapy in various cancer types and have the potential to be used as an anti-cancer agent. In the following subtopics, investigations on Chlorella effects against various types of cancer-based on were elaborated and the overall background of Chlorella was explained.

Chlorella

Chlorella is a eukaryotic microalga that falls under Chlorophyta or known as the green algae group. It is one of the most important genera under the group, as it portrays many biological compounds that have medical importance. It is spherical to ovoid (Figure 1) with a microscopic size of 2-10 μm diameters and lack of flagella (non-motile).

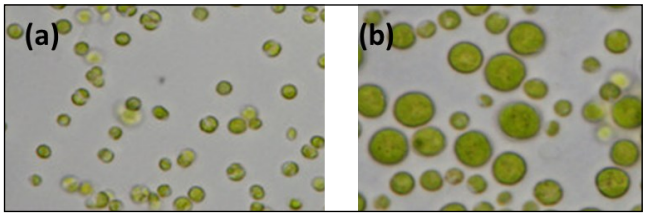

Figure 1: (a) Chlorella sorokiniana (b) Chlorella zofingiensis[8]

The primary composition of Chlorella is proteins (51%-58%), lipids (14%-22%), and carbohydrates (12%-17%); and other elements in Chlorella include vitamins, minerals, and pigments. Proteins in Chlorella have various functions with almost 20% of them bound to the cell wall, more than 50% are in the cytoplasm and 30% migrate in and out of the cell.[9]

Chlorella can synthesize essential and non-essential amino acids and because of this, they are used in human and animal nutrition.[10] In Chlorella, there are two main types of lipids which are polar lipids (glycolipids and phospholipids) and nonpolar lipids or neutral lipids (mono-, di-, and triacylglycerols) as well as small amounts of free fatty acids.[11] While carbohydrates in Chlorella are mainly starch which can be found in the chloroplast, another is cellulose that made the cell wall.[12] In the cell wall of Chlorella, a mix of sugars such as rhamnose, galactose, glucose, xylose, arabinose, and mannose can also be found.[13] In *Chlorella vulgaris*, important vitamins such as Vitamin A, E, C, and B complex were detected. Macro- and microminerals are found also in Chlorella[14] with potassium, sodium and phosphorus are higher than other minerals. Chlorophyll is the most abundant pigment in Chlorella as it functions to absorb light for photosynthesis.[15] Also, Chlorella also possesses accessory pigments for a similar function and they include various carotenoids (Table I) that have numerous health potential benefits.

Table I: Pigment content in *C. vulgaris*[15]

Pigments	Dry Weight (μg g^{-1})
B-Carotene	7-12,000
Astaxanthin	550,000
Canthaxanthin	362,200
Lutein	52-3830
Chlorophyll-a	250-9630
Chlorophyll-b	72-5770
Pheophytin-a	2310-5640
Pheophytin-b	N/A
Violaxanthin	10-37

Due to its nutritional composition, water extract of Chlorella is made into "Chlorella Growth Factor" as the Chlorella water extract contains various components such as nucleic acids, amino acids, vitamins, minerals, polysaccharides, glycoproteins, etc.[16] Because Chlorella also contains carotenoids such as α- and β-carotenes, lutein, zeaxanthin, violaxanthin, neoxanthin. canthaxanthin and astaxanthin, several species of Chlorella have been proposed and extensively studied to be a producer for lutein and astaxanthin.[17,18]

Both lutein and astaxanthin has been known for its antioxidant property and may contribute in prevention of many diseases including cardiovascular, inflammatory, and cancer diseases. Intake of Chlorella itself and its extract has been shown to enhance immune functions[19], reduce cholesterol and hypertension[20, 21], improve

ulcerative colitis[22], and prevent from tumour and cancer diseases[23].

Effect of Chlorella on Cancer

Numerous studies have shown that microalgae found in both fresh and sea waters contain compounds that are potent to several types of cancer.[24] Out of all microalgae, a growing number of studies investigated Chlorella for its potential to treat cancer as compared to other eukaryotic microalgae owing to its various nutritional composition, rapid growth, and resilience in suspension culture. In this article, 68 experimental or original articles are reviewed but only 22 of the most significant observations on Chlorella effects against cancers are discussed here. The types of Chlorella extract and the methodology of treatment adopted in the 22 articles are shown in Table II.

Breast Cancer

Early investigation on the effect of Chlorella against breast cancer was carried out by administrating heat extracted of *Chlorella pyrenoidosa* orally and intraperitoneally before mice were transplanted with mouse mammary carcinoma cells (MM-2).[25] This investigation demonstrated that up to 80% of the mice survived more than 60 days compared to control (tumour bearing mice) that died within 20 days. Another study showed *C. vulgaris*-treated tumour bearing mice were alive up to 45 days while the control died within 20 days when the *C. vulgaris* was given after the cancer treatment.[26] In this study, myelosuppression observed in tumour bearing mice indicated by decreased numbers of bone marrow and spleen granulocyte-macrophage progenitor cells (CFU-GM) was restored after administration of Chlorella. This suggests that the anticancer effect of Chlorella extract may at least in part, attribute to the enhancement of the extract to produce and maturate granulocytes and macrophages. A more recent study demonstrated that the restoration of the reduced number of CFU-GM cells in Ehrlich bearing mice by *C. vulgaris* cell fed is because the extract increased the production of IL-6 and IL-1α.[27] Also, the *C. vulgaris* fed enhanced natural killer cell activity and production of IL-2, IFN-γ, and TNF-α that were reduced in the tumour bearing mice. These could have led to the anti-breast cancer effect of the Chlorella extract. [27]

Another study showed providing *C. pyrenoidosa* powder to the diet of breast cancer-induced rats (by treating with N-methyl-N-nitrosourea) suppressed tumour frequency up to 61% and lengthened tumour latency up to 12.5 days compared to non-treated rats.[28] This suppressed tumour activity may be explained due to the elevated expression of apoptosis executioner protein, caspase-7, and reduced expression of vascular endothelial growth factor receptor-2 (VEGFR2) which is responsible for degrading matrix components for migration and proliferation of cells after Chlorella diet treatment was given. [28] The inhibitory effect of Chlorella against breast cancer could also because of the inhibition of MMP-1 at mRNA and protein levels through c-Jun down-regulation.[29] MMP-1 which is responsible for degrading matrix components for migration and proliferation of cells was found to be over-expressed in breast cancer.[30] Similarly to c-Jun which was also over-expressed in breast cancer.[31] Thus, downregulation of both MMP-1 and c-Jun by Chlorella could have led to the negative effect against breast cancer.

In the most recent study, the capability of Chlorella to generate oxygen via photosynthesis was used to treat a hypoxic tumour in mice. *C. vulgaris* was incorporated in BSA-gel which contains ultrasmall gold nanorods (AuNRs).[32] This Chlorella-gold gel complex was later transplanted within the xenografted 4 T1 breast tumors and improved the hypoxic state of the tumour after exposure to 660-nm light. The presence of oxygen via an increase in oxy-hemoglobin level in hypoxic tumors of mice improved the effect of doxorubicin (dox) to kill the tumour compared to dox-treated mice without the Chlorella-gold gel complex. While the presence of gold nanorods in the gel complexes increases the temperature of immediate vicinity upon 800-nm light and led to the expansion of tumour vasculature which enhances the delivery of dox to kill cancer.

Liver cancer

Chlorella has been reported to exert negative effects against liver cancer. It was observed in a study carried

Table II: The types of Chlorella forms and; *in vitro* and *in vivo* methodology of Chlorella treatment

Types of Cancer	Chlorella species	Types of Chlorella	Treatment Method	References
Breast	*Chlorella pyrenoidosa*	- Non-treated Chlorella - Autoclaved Chlorella - Hot water extracts of Chlorella (Chlorella extract by hot water) - Cell wall fraction of Chlorella	Oral and intraperitoneal treatments of extract at 1 mg/animal of different types of Chlorella were administered to the respected group of mice before mouse mammary carcinoma cells (MM-2) were transplanted to the mice. Treatment for every other day from 7 to 2 days before the tumor transplantation.	25
	C. vulgaris	Dry *C. vulgaris* (CVE) obtained from Chlorella Industry Co Ltd (Tokyo, Japan) was dissolved in distilled water	Oral administration of 50, 100, or 200 mg/kg CVE to Ehrlich ascites tumor-bearing mice for 5 consecutive days.	26
	C. vulgaris	Dry *C. vulgaris* provided by Chlorella Industry Co Ltd (Fukuoka, Japan) was dissolved in distilled water	Daily oral treatment of 50 mg/kg Chlorella to Ehrlich ascites tumor-bearing mice starting 10 days before tumor inoculation and being extended for periods of 3, 8, or 13 days after tumor inoculation.	27
	C. pyrenoidosa	*C. pyrenoidosa* powder	Chlorella powder was mixed with diet at concentrations of 0.3% and 3% and fed to mice one week before carcinogen (N-methyl-N-nitrosourea) administration and until 14 week after carcinogen administration.	28
	C. minutissima	Protein extract of *C. minutissima* (extract obtain after hot alkali treatment to cell pellet)	Protein extract of *C. minutissima* at 10-25 µg/100 µl were treated to MDA-MB 231 and HepG2 cells for 24 hrs.	29
	C. vulgaris	Gelling BSA-PEG–based hydrogel containing Chlorella and gold nanorods (Chlorella AuNRs BSA-Gel)	Injection of Chlorella AuNRs BSA-Gel near to the tumor site in mice until the 20th day. The mice were xenografted with 4 T1 breast cancer cells.	32
Liver	*C. vulgaris*	*C. vulgaris* pellet	Daily force-feeding of *C. vulgaris* pellet at 50, 150, and 300 mg/kg body weight to liver cancer-induced rats for 12 weeks.	33
	C. vulgaris	*C. vulgaris* pellet	Daily force-feeding of C. vulgaris pellet at 50, 150, and 300 mg/kg body weight to liver cancer-induced rats for 12 weeks.	35
		C. vulgaris hot water extract	*C. vulgaris* water extract at 0.1-4 mg/ml was treated to HepG2 cells for 24 hrs.	
	C. vulgaris	*C. vulgaris* water extract	C. vulgaris water extract at 0.1-4 mg/ml was treated to HepG2 cells for 24 hrs.	36
	C. vulgaris	*C. vulgaris* pellet	Daily force-feeding of C. vulgaris pellet at 50, 150, and 300 mg/kg body weight to liver cancer-induced rats for 12 weeks.	37
	C. vulgaris	Hot water extract of *C. vulgaris* which was isolated in Malaysia and Japan	*C. vulgaris* water extract at 0.1-4 mg/ml was treated to HepG2 cells for 24 hrs.	38
	C. vulgaris	*C. vulgaris* (purchased at Puli, Nantou, Taiwan) aqueous extract	*C. vulgaris* aqueous extract at total phenolic content 30-90 µg was treated to HepG2 cells for 72 hrs.	39
	C. minutissima	Protein extract of *C. minutissima* (extract obtain after hot alkali treatment to cell pellet)	Protein extract of *C. minutissima* at 10-25 µg/100 µl were treated to MDA-MB 231 and HepG2 cells for 24 hrs.	29
Colorectal	*C. vulgaris* *C. ellipsoidea*	- Semipurified extracts of *C. vulgaris* containing mostly carotenoids - *Semipurified extracts of C. ellipsoidea* containing mostly carotenoids	Semipurified extracts of *C. vulgaris* and *C. ellipsoidea* at 5- 100 µg/ml were treated to HCT116 cells for 24 hrs.	40
	C. protothecoides	- *C. protothecoides* oil extract encapsulated within polymer micro -particles - non-encapsulated *C. protothecoides* oil	Human glioblastoma cells (A172), and human colorectal cancer cells (HCT-116) were treated with 17.5, 35, and 70 µM chlorella oil microparticles and non-encapsulated *C. protothecoides* oil for 48 hrs.	41
	C. zofingiensis *C. vulgaris*	Exopolysaccharide (EPS) of *C. zofingiensis and C. vulgaris*	HCT8 cells were exposed with EPS of *C. zofingiensis and C. vulgaris* at 0.15, 0.3 and 0.6 mg/ml for 24 hrs.	42
	C. pyrenoidosa	Exopolysaccharide (EPS) of *C. pyrenoidosa*	HCT8 or HCT116 cells were exposed with EPS of *C. pyrenoidosa* at 0.15, 0.3, and 0.6 mg/ml for 24 hrs.	43

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Lung	*C. vulgaris* C-C	Supercritical carbon dioxide extract of *C. vulgaris*	Supercritical carbon dioxide extract of *C. vulgaris* at 200, 100, and 20g/ml was exposed to non-small cell lung cancer (NSCLC), H1299, A549, and H1437 cells for 24 hrs.	44
	C. sorokiniana	*C. sorokiniana* (provided by International Cryptomonadales Biotechnology, Taiwan) water extract *C. sorokiniana* was dissolved in double distilled water	*C. sorokiniana* water extract at 0–1000 ng/ml was treated to NSCLC cell line, A549, and CL1-5 for 24 hrs. *C. sorokiniana* oral intake at 50 mg/kg body weight of mice bearing tumor (CL1-5 cells injected subcutaneously in the right flank of mice). Oral intake was carried out for 11 days.	45
Lymphoma	*C. sorokiniana*	*C. sorokiniana* methanol extract	*C. sorokiniana* methanol extract at 7.8, 15.6, 31.2, 62.5, 125, 250 and 500 µg/ml was exposed to murine L5178Y-R lymphoma cell line and normal lymphocyte cells for 24 hrs.	46
Cervix	*C. pyrenoidosa*	Organic extract (dichloromethane/ methanol; 2:1) of *C. pyrenoidosa* (CP)	Pre-coated coverslips containing vascular endothelial growth factor (VEGF) and CP extracts at 25, 50, and 100 µg were placed over the developing chorionic allantoic membrane of fertilized chicken eggs for 12 days. CP extract at 7.8, 15.6, 31.2, 62.5, 125, 250 and 500 µg/ml was exposed to HeLa cells for 24 hrs.	47
Stomach	*C. vulgaris*	Peptide fraction isolated from pepsin hydrolysate of *C. vulgaris* protein waste	Peptide fraction of *C. vulgaris* was exposed to stomach cancer cells AGS, human colon adenocarcinoma cells C2BBel, human hepatoblastoma cell lines Hep G2, and human cervical epithelioid carcinoma cells, Hela for 24 hrs.	48
Prostate	*C. marina*	- lycopene (cis and trans 60:40) isolated from *C. marina* (CL) - Tomato lycopene (CL)	PC-3 and DU-145 cancer cells were treated with AL and TL (20 and 50 µM, respectively) for 24 hrs	49

out Sulaiman et al.33 that supplementation of C. vulgaris diet reduced the number of preneoplastic liver nodules formed in ethionine-induced liver carcinogenesis rats. They also found that antioxidative enzymes, superoxide dismutase (SOD), and catalase that were observed to be high in ethionine-induced liver carcinogenesis rats were low when *C. vulgaris* diet was fed to these rats. This confirmed the *C. vulgaris* effect against liver cancer as SOD and catalase that are responsible for eliminating excessive free radicals is low when given *C. vulgaris* diet to the liver cancer-induced rats. Sulaiman *et al.*[33] suggested that the *C. vulgaris* effect against liver cancer maybe through the reduction of lipid peroxidation as they found that the increase MDA level in liver cancer-induced rats was reduced when fed with *C. vulgaris* diet. Increase lipid peroxidation in the body has repeatedly shown to lead to the formation of cancer.[34]

A group of researchers demonstrated that high doses of *Chlorella vulgaris* at 300 mg/kg/day in a rat diet not only allow preneoplastic tumor in the liver to shrink to 83% of the initial tumors but also suppress tumor proliferation.[35] Several explanations were reported with regards to the tumours shrinkage and suppression in the liver. The first plausible explanation is that *Chlorella vulgaris* aqueous extract induces apoptosis by lowering the expression of the anti-apoptotic protein, Bcl-2 which is associated with hepatocyte proliferation but increases the expression of pro-apoptotic protein, caspase 8 that correlates with increased apoptosis.[36] More recent study reported the ability of *Chlorella vulgaris* to downregulate the expression of liver tumor markers in liver tissue such as M2-pyruvate kinase (M2-PK), alpha-fetoprotein (AFP) and a specific antigen for oval cells (OV-6) as well as transforming growth factor-β (TGF- β) in serum.[37]

In vitro studies showed hot water extract of *Chlorella vulgaris* possessed an antiproliferative effect against

HepG2 liver cancer cells, with an IC_{50} of 1.6 mg/mL.[38] It is important to note that high IC_{50} is acceptable as *Chlorella vulgaris* is classified as food and not a drug. Further tests on HepG2 liver cancer cells showed *Chlorella vulgaris* aqueous extract at 2 mg/mL caused 70% apoptosis of HepG2 cells compared with normal liver cells, WRL-68 which showed 15% apoptosis when treated at the same dose. A higher apoptotic percentage of HepG2 liver cancer cells can be elucidated by the inhibition of DNA synthesis which eventually caused DNA damage.[38] Of the many reasons, the antioxidant properties of *Chlorella vulgaris* have been a key feature that induces apoptosis in activated hepatic stellate cells (HSC) and thereby ameliorate liver fibrosis.[39] Detailed mechanism of apoptosis in HepG2 liver cancer cells has been further described by Yusof *et al*[36] that a series of signaling cascades are involved in the apoptotic pathway which includes increased expression of pro-apoptotic proteins such as p53, Bax, and caspase-3 and decreased expression of anti-apoptotic protein Bcl-2. Besides inhibiting the proliferation of cancer cells, Chlorella also can prevent the tumor from metastasizing. During tumor development, basement membrane disruption is an essential step for tumor invasion and metastasizes. This condition occurs due to the highly expressed extracellular matrix metalloproteases MMP-2 and MMP-9. Protein extract of *Chlorella minutissima* was observed to suppress the overexpression of metalloproteases MMP-2 and MMP-9 in HepG2 cells.[29] Considering the potential of Chlorella in reducing further damage caused by free radicals, suppressing tumor proliferation, and preventing cancer cells from metastasizing, it can be suggested that Chlorella has high potential in cancer remedies.

Colorectal Cancer

Microalgal carotenoids have been used in the treatment of colorectal cancer combined with the chemotherapeutic agent 5-fluorouracil to facilitate complete remission.[24] Previous study showed carotenoids extracted from *Chlorella ellipsoidea* and *C. vulgaris* were cytotoxic to human colon cancer cells, HCT116.[40] Carotenoids from both species were also found to exert apoptosis-inducing effect to the colon cancer cells with *C. ellipsoidea* carotenoids showing 2.5 times stronger effect than *C. vulgaris* carotenoids. Carotenoids responsible for this activity might be violaxanthin as HPLC analysis revealed that violaxanthin is the main carotenoid found in *C. ellipsoidea*.

Chlorella protothecoides oil extract encapsulated within polymer micro-particles also showed a cytotoxic effect on similar colon cancer cell line, HCT116.[41] Having polymer encapsulating the microalgal oil does not only protect the algal bioactive compounds but it also allows expression of comparable cytotoxicity effect that stimulated by non-encapsulated microalgal oil. Other than microalgae intracellular compounds, many studies also focused on the production and anti-cancer properties of microalgal exopolysaccharide (EPS). The EPS extracted from *C. pyrenoidosa*, *C. zofingiensis,* and *C. vulgaris* at a concentration of 0.6 mg/mL showed significant inhibitory effects at 35.9, 28.3, and 18.0%, respectively against human colon cancer cell lines HCT8.[42,43] The inhibition activity against tested colon cancer cell lines indicates that EPS from Chlorella sp. may be effective agents for anti-cancer therapeutics.

Others

Other than significant effects of Chlorella on breast, liver, and colon cancers; multiple studies had been conducted to study the effect of Chlorella on other types of cancers. Several studies had been carried out to investigate the effects of *Chlorella sp.* on non-small cell lung cancer (NSCLC) which is the most common type of lung cancer that usually starts from the outer part of the lungs. It has been shown that *C. vulgaris* extract obtained using supercritical carbon dioxide extraction (SC-CO_2) method inhibited NSCLC cell lines, H1299, A549, and H1437 cells in a dose-dependent manner.[44] In the same study, they demonstrated the extract significantly decreased the migration of all tumor cells, suggesting that *C. vulgaris* extract may inhibit metastasis of lung cancer. The exact bioactive compounds responsible for these activities, however, were not identified in this study nevertheless they found that the extract has a high content in polyphenolic and flavonoid compounds. *Chlorella sorokiniana* was also found to inhibit the growth of NSCLC cell line, A549 as well as CL1-5 in a different study.[45] *In vivo* study carried out by Lin *et al*[45] showed tumor growth of CL1-5 cells injected

subcutaneously in the right flank of mice was markedly inhibited after oral intake of *C. sorokiniana*. They showed that the effect of *C. sorokiniana* against NSCLC cells is through induction of mitochondrial-mediated apoptosis by downregulation of Bcl-2, XIAP, and survivin.

In recent study, *C. sorokiniana* methanol extract was tested against lymphoma cells, L5178Y-R, and was demonstrated to cause 61% tumor cell toxicity at 500 mg/mL.[46] For this cytotoxic effect, *C. sorokiniana* causes apoptosis towards the lymphoma cells however it was not through the caspase-3 activity. Chlorella also showed a negative effect on cervix cancer cells when organic extract (dichloromethane/methanol; 2:1) of *Chlorella pyrenoidosa* was found to inhibit the growth of HeLa cells at IC_{50} of 570 µg/ml.[47] As *C. pyrenoidosa* was also shown to be antiangiogenic in the same study[47] (Figure 2), preventing the growth of new blood vessels may indirectly contribute to the fight against cervix cancer and maybe all types of cancer.

A study on identifying the substance that is responsible for the anticancer effect of *C. vulgaris* against adenocarcinoma stomach cancer cells, AGS demonstrated that *C. vulgaris* peptide fraction with low molecular weight had strong dose-dependent cytotoxic activity and stimulated cell cycle arrest at post-G1 in AGS cells.[48] The peptide fraction possesses cell-specific effect as no significant inhibitive effect was observed against human colon adenocarcinoma cells C2BBel, human hepatoblastoma cell lines Hep G2, and human cervical epithelioid carcinoma cells, Hela. Among the peptides in the fraction, a short peptide with an amino acid sequence, VECYGPNRPQF showed effective antiproliferative, antioxidant, and anti-inflammatory activities. Comparison investigation on lycopene extracted from *Chlorella marina* and tomato showed that algal lycopene (AL) inhibited the growth and colony formation of prostate cancer cells, PC-3 and DU-145 more effectively than tomatal lycopene (TL).[49] AL also induced apoptosis and arrested the cell cycle at the G_0/G_1 phase at a higher level than TL. The authors suggested that AL showed a higher effect than TL may be due to the presence of a mixture of cis- and trans-lycopene in AL while only trans-lycopene was found in TL. It has been demonstrated that cis-lycopene is easily absorbed and possess higher antioxidant activity compared to all-trans lycopene.[50]

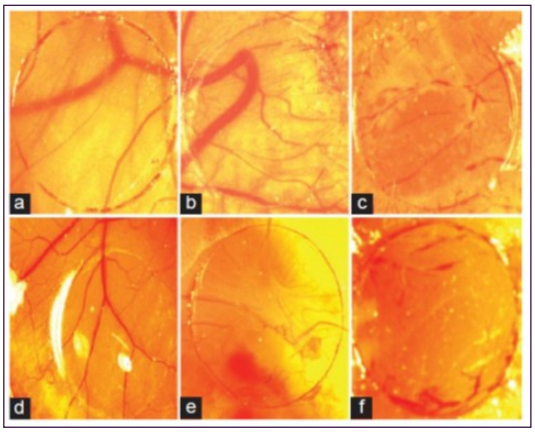

Figure 2: Indicated the chorioallantoic membrane (CAM) of a 12-day old chick embryo incubated for 4 days with a coverslip treated with or without treatment. (a) Normal, without treatment; (b) Treated with vascular endothelial growth factor, VEGF (50 ng); (c) Treated with VEGF (50 ng) + thalidomide (10 µg); (d-f) Treated with VEGF (50 ng) + *C. pyrenoidosa*, CP at the concentrations of 25, 50, and 100 µg, respectively. CAM treated with VEGF showed profuse growth of blood vessels while treatment with VEGF + CP extracts demonstrated decreased blood vessel density in a dose-dependent manner.[47]

CONCLUSION

Chlorella is photosynthetic eukaryotic microorganisms that possess diverse effects on health benefits in both humans and animals. Owing to its intracellular and extracellular compounds, Chlorella has strong potential in cancer treatments and reduces the side effects of conventional anticancer agents. To date, Chlorella either in powder, crude extract form, or specific compounds demonstrated potent effects against various types of cancer. Its consistent effects on cell cycle arrest, apoptosis of cancer cells, downregulating components involve in migration and proliferation of cells as well inhibiting angiogenesis play important roles in inhibiting cancer growth.

Other than that, Chlorella also stimulates the production and maturation of granulocytes and macrophages *in vivo* that could lead to the anti-cancer effect. As of now, Chlorella is widely used as supplementary after conventional cancer treatment as well as for general health purposes. Although Chlorella's effects against cancer are not as potent as conventional chemotherapy agents, daily consumption of Chlorella will at least halted the growth of potential cancer cells after cancer patients stop receiving their treatments.

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