REVISITING THE ROLE OF PHYCOCYANIN IN CURRENT CLINICAL PRACTICE

A. Soni 1, M. Dubey 2, M. Verma 1, R. Dhankhar 1, V. Kaushal 1, R. Atri 1 and R. Sabharwal 3

Department of Radiotherapy 1, Department of Biochemistry 2, PGIMS, Rohtak, Haryana, India. Consultant Radiation Oncologist 3, Department of Health, Government of Haryana, Kurukshtra, Haryana India.

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**Correspondence to Author:**
Dr. Abhishek Soni
Senior Resident, Department of Radiotherapy, Post-graduate institute of medical sciences, Rohtak, India – 124001

**E-mail:** abhisheksoni246@gmail.com

**ABSTRACT:** C-phycocyanin (C-PC) is a biliprotein found in edible blue-green algae. Its anti-cancer, anti-inflammatory, anti-proliferative and anti-oxidant activities has been evidenced by many in vitro and in vivo studies. Anti-inflammatory activity of C-PC demonstrated by free radical scavenging, inhibition of DNA damage, and decreased ROS. Here, the effects of C-PC on cytotoxicity and cell signaling through the apoptotic and other pathways were studied. Results indicated that anti-proliferative effects of C-PC are mediated by inactivation of BCR-ABL signaling and the downstream pathway PI3K/AKT. Phycocyanin induces apoptotic death, and Bcl-2 expression inhibits it via generation of free radicals. C-PC exerted antimelanogenic mechanisms by downregulating p38 MAPK-regulated CREB pathway activation and upregulating MAPK/ERK-dependent degradation of MITF protein. C-PC-mediated PDT (photodynamic therapy) is approved as a potential therapy for cancer. Phycocyanin can be consumed as a dietary supplement or a food component to obtain health benefits against CVD (cardiovascular disease) and NAFLD (non-alcoholic fatty liver disease). These results suggest that phycocyanin which had been obtained from blue green algae are potential medicaments in the treatment of various kinds of human ailments and cancers.

**INTRODUCTION:** Phycocyanin extracted from Spirulina was first marketed in 1980 by Dainippon Ink and Chemicals under the brand name “Lina Blue–A.” Phycocyanin is a dietary supplement dedicated to individuals who are undergoing chemotherapy and radiation for cancer and is used to ease negative symptoms during treatment as well as rejuvenate post treatment.

Phycocyanin is an important molecule extracted from Spirulina platensis, a 3.6 billion years old blue green algae and its nutritional values and therapeutic values are well documented. Phycocyanin is a water soluble, natural and non-toxic molecule with anticancer, antioxidant, antiviral and anti-inflammatory activities. Phycocyanin also has neuroprotective profile of phycocyanin. Spirulina is known to have nutritional advantages of high-quality protein, minerals, vitamins, essential amino acids, cyanocobalamin (B12), tocopherols and essential fatty acids including β-carotene and γ-linolenic acid (GLA). Phycocyanin is also a powerful agent for the immune system in human and animals, and provides protection from a number of diseases. The deep blue colour of Phycocyanin has been widely used as a colorant in food industry for food additive purposes. Various research studies also support strong hepatoprotective, cytoprotective and neuroprotective profile of phycocyanin.

Most of the studies are based on the laboratory experimental results on mice and cultured cell lines, and, still very less literature is available to conclude any concrete result for Phycocyanin use.
in current era of medical practice. Thus, this article reviews the role of phycocyanin in today’s clinical practice.

**Structure:**
Phycocyanin occurs as the major phycobiliprotein in Cyanobacteria and as a secondary phycobiliprotein in some red algae. C-Phycocyanin (C-PC) is a natural compound. The pigment is composed of two subunits, α and β, which occur in equal numbers, but the exact number may vary among the species. Both α and β subunits contain only the PCB chromophore. The structure of C-phycocyanin from the thermophilic blue green algae Mastigocladus laminosus has been determined at 3 Å resolution by X-ray diffraction methods. The protein found to consist of three α-β units arranged around a threefold symmetry axis to form a disc with dimensions of 110 Å x 30 Å with a central channel of 35 Å in diameter. Both subunits, α and β, have a similar structure and are related by a local twofold rotational axis. Its molecular weight is between 70,000 and 110,000 Daltons. Phycocyanin has visible absorption maximum between 615 and 620 nm and maximum fluorescence emission at ~650 nm. Phycocyanin accepts quanta from phycoerythrin by fluorescent energy transfer in organisms in which it is present.

The purity of C-PC is evaluated using the absorbance ratio of A620/A280, and a purity of 0.7 is considered as food grade, 3.9 as reactive grade, and more than 4.0 as analytical grade. Purity is directly related to process costs, and, in general, the more purified a product is, the more expensive to obtain.

**Sources:**
Phycocyanin can be extracted mainly from blue green algae and may be from red algae (rhodophytes) and cryptophytes. Main sources are Spirulina platensis, Arthrospira platensis, Spirulina maxima, Phormedium fragile, Nostoc muscorum, Oscillatoria species, Nostoc humifusum, Anabaena oryzae, Wollea saccata, Anabaena flous aquae, Limnothrix and Porphyra haitanensis, thermophilic cyanobacterium Mastigocladus laminosus, Agmenellum quadruplicatum, Remyella diplosiphon, Phanizomenon flos-aqueae, marine cyanobacterium Synechococcus sp. IO9201, Galdieria sulphuraria (rhodophyte), Anabaena sp. PCC 7120, Leptolyngbya species, Scytonema julianum, Thermosynechococcus elongates, Tolypothrix tenuis, Nostoc minutum, Klamath algae, Acaryochloris marina, and Microcystis aeruginosa.

**Extraction and Purification:**
A number of drying methods like spray dried, crossflow dried and oven dried methods, are used for Spirulina processing, and results in approximately 50% loss of phycocyanin. So fresh biomass is suitable for extraction of phycocyanin. Blue green algae are grown in nutrient medium (like Zarrouk’s medium) containing different amounts of nitrogen and salt. In Spirulina species, Phycocyanin pigments increase from 12% to 22% on increasing nitrogen levels. Spirulina has a wide variety in composition of Phycocyanin pigments ranging from R-phycocyanin (R-PC) from 5.75 to 12.35%, allophycocyanin (A-PC) from 2.53 to 6.11% and C-phycocyanin (C-PC) from 1.65 to 4.02% as a result of changing nitrogen contents and salt stress.

The concentration of Phycocyanin pigment including C-Phycocyanin (C-PC), allophycocyanin (A-PC), and R-phycocanin (R-PC) are determined spectrophotometrically at 618 and 650 nm, 650 and 618 nm, and 498, 615 and 650 nm respectively as reported by Kursar and Alberte. Cyanobacteria adjusts the contents and relative ratio of its pigments with the light quality, like more production under red or violet light. C-PC is a natural blue pigment accounting for 14% of SP dry weight. The increase in NaCl levels in nutrient...
medium led to significant increase in production of Phycocyanin contents and soluble protein in Spirulina cells. Phycocyanin production also depends on iron concentration in the media. Thus, Spirulina species grown under combined stress of high NaCl and nitrogen deficient levels, produce higher amount of Phycocyanin.

Many methods have been used for the separation and purification of C-PC. C-PC is purified from Spirulina platensis by ammonium sulfate precipitation, ion-exchange chromatography, modified flow cytometry and free-flow electrophoresis (FFE). SDS-polyacrylamide gel electrophoresis is performed to assess the molecular weight and purity of C-PC. SDS-PAGE analysis of the purified fraction clearly showed 2 protein bands corresponding to α and β subunits of C-PC. The purity of C-PC, as judged by an A620/A280 purity ratio greater than 4.0, is sufficient for further testing. Femtosecond laser spectroscopies are used to examine the electronic structures of cyanobacteria.

Table 1 shows the purity of C-PC after various stages of purification which can be implied for further analysis of grading the phycocyanin. The table signifies that the purity can be increased and a food grade phycocyanin was isolated.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Purification process</th>
<th>Extraction purity at 615/280nm</th>
<th>Phycocyanin concentration (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Crude Phycocyanin</td>
<td>0.61</td>
<td>1.94</td>
</tr>
<tr>
<td>2</td>
<td>Ammonium sulphate precipitation</td>
<td>0.73</td>
<td>2.39</td>
</tr>
<tr>
<td>3</td>
<td>Membrane filtration</td>
<td>0.89</td>
<td>2.64</td>
</tr>
<tr>
<td>4</td>
<td>Dialysis</td>
<td>0.99</td>
<td>2.90</td>
</tr>
</tbody>
</table>

Cell cytotoxicity was evaluated by the 2,3-bis[2-methoxy-4-nitro-5-sulfophenyl]-2H-tetrazolium-5-carboxyanilide inner salt (XTT) assay. Western blot analysis is used to study the expression and phosphorylation of proteins in the PI3K/AKT pathway. The oxygen radical absorbance capacity (ORAC) assay is used to assess antioxidant activity of phycocyanin. C-PC concentration from G. sulphuraria is low than cyanobacteria.

**Uses:**
The spray-drying method is used to load chitosomes with C-phycocyanin for colonic drug delivery. Phycocyanin extracted from Spirulina platensis displayed favorable health benefits such as improving immune function, promoting zooblast regeneration and inhibiting the growth of cancer cells. Since phycocyanin is photosensitive, it has been proposed as a new photosensitizer for photodynamic therapy. C-phycocyanin is endowed with various biological and pharmacological properties. C-phycocyanin possesses significant antioxidant activities and can enhance immunity and inflammatory responses.

Studies have shown that C-phycocyanin can induce apoptosis in cancer cells such as a mouse macrophage cell line (RAW 264.7), prostate cell line (LNCaP), breast cell line (MCF-7), and erythromyeloid leukemia cell line (K562). Phycocyanin is used in treatment of hepatocellular carcinoma, rectal cancer, leukemia, melanoma and used in food industry, biomedicine and cosmetics industry. Subashini et al demonstrated that C-phycocyanin induced apoptosis in K562 cells is mediated by cytochrome c release, PARP cleavage, and Bcl-2 down-regulation. C-phycocyanin could be used for treating ischemia-reperfusion injury through the activation of ERK pathway and suppression of p38 MAPK pathway. Phycocyanin is a potent antioxidant as well as herbical agent, and possesses significant immune enhancing and antiviral properties. It’s enhancing biological defense activity against infections disease reduces allergies inflammation by the suppression of IgE antibody.

The phycocyanin is used as coloring agent in food item like jellies, chewing gums, ice sherbaths, and dairy products, and it enhances antioxidant capacity of food and beverages. In Japan, China, and Thailand, phycocyanin is used in cosmetics like lipstick and eyeliners. It is used in biomedical research and pharmaceutical industries. It is used in immune diagnostic applications. C-PC selectively stimulates the lymphocyte antioxidant defence system of occupationally exposed subjects. The activation of the antioxidant protective mechanisms as part of the early radiation response.
is probably related to the chronic low-dose occupational exposure. The modulating capacity of C-PC at the molecular level may be of interest for the protection of occupationally exposed persons.\textsuperscript{44}

**Antioxidative Properties of Phycocyanin:**
Antioxidant activity of Phycocyanin is demonstrated by ascorbate/iron/H2O2 assays, DPPH (2,2 diphenyl-1-picrylhydrazyl) assay and ABTS (2,2'–azino-bis ethylbenzthiazoline-6-sulfonic acid) assay.\textsuperscript{3, 45} Oxidative stress, mainly characterized by reactive oxygen species (ROS), damages tissues and therefore is associated with several pathological conditions, such as atherosclerosis, nonalcoholic steatohepatitis (NASH), and aging.\textsuperscript{46} Hydroxyl and peroxyl radicals are associated with oxidative damage to lipids and DNA.\textsuperscript{46} Human studies have reported the protective effects of BGA against oxidative damage in vivo and in vitro.

SP supplementation of 8 grams per day for 12 weeks significantly decreased plasma levels of MDA (malondialdehyde), a biomarker of oxidative stress, in diabetic patients. Similarly, healthy elderly Korean subjects who consumed 8 grams per day of SP for 16 weeks showed a decrease in the lipid peroxidation level, whereas the total antioxidant status and levels of antioxidant enzymes, such as SOD and GPx, were elevated in plasma, indicating that SP supplementation was able to improve antioxidant status in the human subjects. C-PC markedly inhibited the production of alkoxyl radicals that are generated by the reaction of tert-buty1 hydroperoxide with ferrous ions in the presence of luminol. In addition, C-PC prevented DNA damage and scavenged hydroxyl and peroxy radical radicals. Furthermore, C-PC inhibited peroxy radical-induced oxidative hemolysis and lipid peroxidation in normal human erythrocytes.\textsuperscript{46, 47}

C-PC from AFA extract demonstrated protective effects against cupric chloride-induced lipid oxidation in human plasma samples. Cysteine-rich cyanopeptide beta 2 isolated from C-PC of SF demonstrated free radical scavenging, inhibition of DNA damage, and decreased ROS production.\textsuperscript{48} Selenium-containing allophycocyanin (Se-APC) extracted from selenium-enriched SP inhibited 2,20-azobis-2-methylpropanimidamide, dihydrochloride (AAPH)-induced oxidative hemolysis, and morphological changes in human erythrocytes.\textsuperscript{49} Se-APC, furthermore, inhibited AAPH-induced intracellular ROS production and MDA accumulation.\textsuperscript{46} Treatment with C-Pc prevents the rats from Tributyltin (TBT) induced thymic atrophy, but not proved in humans for such a role.\textsuperscript{50} Phycocyanin may inhibit atherosclerosis by activating heme oxygenase-1.\textsuperscript{51} Phycocyanin helps in displacing fluoride, facilitating antioxidant formation, reverses sodium fluoride-induced thyroid changes, improves behaviour and protects Purkinje cells. Phycocyanin supplementation during pregnancy may reduce the risk of fluoride toxicity to offspring.\textsuperscript{52}

**Lipid-Lowering Effect of Phycocyanin:**
In human clinical trials, supplementation with Spirulina Platensis (SP) exhibited lipid-lowering effects. In patients with type 2 diabetes, subjects who consumed 2 grams per day of SP for 2 months showed significantly lower plasma triglyceride (TG) concentrations as well as a significant reduction in ratios of total cholesterol : HDL-C and LDL-C : HDL-C.\textsuperscript{46} Eight grams per day of SP supplementation for 12 weeks significantly reduced plasma triglyceride concentrations and blood pressure in type 2 diabetic patients with higher initial triglyceride levels, whereas subjects with high initial total cholesterol and LDL-C showed significant reductions in the plasma lipids. In patients with a hyperlipidemic nephritic syndrome, 1 gram per day of SP supplementation for 2 months significantly decreased plasma total cholesterol, LDL-C, and triglyceride concentrations. Up to now, BGA dosages used in human clinical trials have ranged from 1 to 8 grams per day for up to 24 weeks, whereas 5 gram per day is generally recommended by the manufacturers.\textsuperscript{46}

Although individual cases of discomfort or unpleasant condition have been reported with BGA supplementation from time to time, Dietary Supplements Information Expert Committee (DSIEC) of the United States Pharmacopeial Convention has awarded Spirulina (SM and SP) a grade A safety rating and agreed that Spirulina is generally safe to be consumed.\textsuperscript{53} Concern over using BGA during pregnancy and breast feeding is
not clearly defined in humans. However, numerous animal studies indicated that SP consumption during pregnancy and lactation did not induce signs of maternal intoxication.\(^{46}\)

In contrast, Kapoor and Mehta had demonstrated that SP supplementation could improve iron status during pregnancy, as evidenced by a higher hemoglobin count, serum iron, and serum ferritin.\(^{54}\)

Further study is necessary to evaluate the effect of BGA supplementation in humans during specific nutrition status and pathological conditions.\(^{46}\)

Morcos et al perfused atherosclerotic artery segments obtained within 5 hours postmortem with 0.1 mg/ml phycocyanin in oxygenated Krebs Ringer solution at 30 mmHg for five minutes followed by washout with Phycocyanin free Krebs for ten minutes. Histologically, on light and fluorescence microscopy, artery section revealed fluorescence localization within the plaque particularly at the site of elastic lamina and also at the internal elastic lamina but not in the medial muscle layer.

These properties suggested therapeutic use of Phycocyanin for plaque localization and regression.\(^{55}\)

Both C-PC and CD59 inhibit the process of atherosclerosis, and the anti-atherosclerotic properties of C-PC might be due to promoting CD59 expression, preventing proliferation of smooth muscle cell and the apoptosis of endothelial cells, reducing lipid levels, and finally inhibiting atherosclerosis.\(^{56}\)

Phycocyanin prevents hypertension and endothelial dysfunction related disease like metabolic syndrome.\(^{57}\)

**Effect of C-PC on Lipid Peroxidation:**

ROS initiate the peroxidation of membrane lipids, leading to the accumulation of lipid peroxides and leakage of cytosolic enzymes into circulation.\(^{46}\)

Bhat and Madyastha reported C-PC inhibited nearly 95% of peroxyl radical-induced lipid peroxidation. C-PC with a reduced chromophore can efficiently inhibit peroxyl radical-induced lipid peroxidation in a dose-dependent manner.\(^{58}\)

**Anti-Inflammatory Effects of Phycocyanin:**

Oxidative stress plays important roles in lung disease, gastrointestinal dysfunction, endothelial dysfunction and atherosclerosis.\(^{45}\) Anti-oxidative and anti-inflammatory effects can be demonstrated by non-alcoholic steatohepatitis model.\(^{59}\)

C-phycocyanin suppresses inflammation by inhibiting the expressions of inducible cyclooxygeanase-2 (COX-2) and nitric oxide synthase, and by inhibiting the production of pro-inflammatory cytokines. Also, Phycocyanin scavenges free radicals, including alkoxyl, hydroxyl and peroxyl radicals; inhibits liver microsomal lipid peroxidation, decreases prostaglandin E(2) production, reduce myeloperoxidase production, decreases nitrite production, inhibits platelets aggregation, and uppresses the activation of nuclear factor-κB (NF-κB) via preventing degradation of cytosolic IkB-α. These all effects lead to anti-inflammatory activity of Phycocyanin.\(^{50}\)

Upto now, the antinociceptive properties of phycocyanin have been less thoroughly investigated.\(^{45}\)

Consumption of BGA has been demonstrated to promote immunity and to protect against inflammatory diseases, such as colitis, arthritis, and allergic rhinitis in animal and human studies.\(^{61}\)

SP organic extracts markedly decreased the secretion of proinflammatory cytokines, including the granulocyte-macrophage colony stimulating factor, IL-6, MCP-1, and TNFa. Moreover, translocation of NF-κB from cytoplasm to nucleus was also inhibited. Anti-inflammatory effects of BGA have been shown in cell studies, animal studies, and human studies. Furthermore, understanding of the effect of BGA on acute or chronic inflammatory pathways is necessary because although the two pathways share some of common mediators, protective effects against chronic inflammation at a low degree for a long period of time are more relevant and beneficial to the prevention of metabolic diseases such as CVD and NAFLD.\(^{46}\)

Several components of BGA, including GLA and PC, have been implicated in their anti-inflammatory effects. SP contains 1.3% GLA. An anti-inflammatory effect of SP is also often associated with antioxidant and antiviral properties of C-PC and R-PC.\(^{46}\)

Phycocyanin supplementation significantly reduces the salicylate-induced tinnitus in mice.\(^{62}\)

**Antibacterial Role:**

Inflammatory Effects of Phycocyanin
The antibacterial activity of S. maxima extracts were assayed against five bacterial strains (P. aeruginosa, B. subtilis, S. aureus, E. coli and Streptococcus sp.) by evaluation of the inhibition of zones. Generally, all S. maxima extracts were found to be effective with antibacterial activity and were dose dependant. This phenomenon was in agreement with that found by Ozdemir et al. The data in Table 2 showed that the most susceptible bacteria were Streptococcus sp. and B. subtilis to S. maxima with highest inhibition zones ranged 6–13 mm at concentrations 100–400 µl/disk. It is of interest to note that all S. maxima extracts manifested similar degrees of susceptible towards both Gram-positive and Gram-negative bacteria. The lower inhibition zone ranged from 6 to 9 mm of extracts obtained for S. maxima.

Table 2: Antimicrobial Activity of Phycocyanin

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Microorganism</th>
<th>Zone of inhibition at various concentrations of C-PC (mm)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>100µl</td>
</tr>
<tr>
<td>1</td>
<td>Streptococcus</td>
<td>9</td>
</tr>
<tr>
<td>2</td>
<td>Pseudomonas</td>
<td>8</td>
</tr>
<tr>
<td>3</td>
<td>Bacillus</td>
<td>8</td>
</tr>
<tr>
<td>4</td>
<td>Staphylococcus</td>
<td>6</td>
</tr>
<tr>
<td>5</td>
<td>E. coli</td>
<td>7</td>
</tr>
</tbody>
</table>

Sarada et al showed that phycocyanin was able to markedly inhibit the growth of drug resistant bacteria Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa and Staphylococcus aureus while no activity was recorded in Acinetobacter baumanii and Enterococcus durans.

Phycocyanin as Immune System Modulator:
The regular intakes of phycocyanin boost immune responses. When used as an adjuvant to chemotherapy Phycocyanin boost immune system to fight cancer spread, improved cancer response to chemotherapy and decreased risk that chemotherapy may give rise to a new cancer. Phycocyanin affects the stem cells in the bone marrow, which produce white blood cells that make the immune system and red blood cells that oxygenate the body. Phycocyanin emulates the affect of erythropoetin, which regulates red blood cells production. Phycocyanin suppresses allergic IgE antibody response and enhances secretory IgA antibody response in mice. The immune cells involved in cancer control, NK cells and cytotoxic T lymphocytes, function more effectively with intake of Phycocyanin.

Phycocyanin Mediated Apoptosis
COX-2 play a significant role in promoting tumor growth in multiple organ systems and is overexpressed in human lung, breast, colorectal and prostate tumors. C-phycocyanin from S. platensis is a specific COX-2 inhibitor. Phycocyanin generates free radicals, which induce damage at macromolecular level, leading to apoptotic cell death. Pardhasaradhi et al demonstrated Phycocyanin inability to induce apoptosis in Bcl-2 transfectants which correlated well with significant decrease in production of ROS (reactive oxygen species) in these cells. Z-VAD, a pan-caspase inhibitor, inhibits phycocyanin-mediated apoptosis, which indicates some role of caspases in apoptotic death of BC-8 cells (monoclonal cells of rat histiocytic tumor). Phycocyanin downregulates Bcl-2 expression in BC-8 cells, thereby make them vulnerable to apoptotic death. Since many tumors are resistant to apoptosis due to Bcl-2 expression, therefore, Phycocyanin treatment leading to Bcl-2 down-regulation, may make them sensitive to other anticancer agents that kill the tumor cells via apoptosis. Pardhasaradhi et al showed that phycocyanin induce apoptosis in tumor cells via ROS production, which is suppressed by Bcl-2.

Anti-Cancer Effects:
Shanab et al showed that Spirulina Platensis showed higher antioxidant activity and moderate anticancer efficiency among various algal species due to its total phycobiliprotein pigments, and secondary metabolites. Shalaby et al revealed that Spirulina platensis shows highest antioxidant activity under salt stress conditions. Wang et al reported that c-phycocyanin interact with the membrane associated glycoldehydes-3-phosphate dehydrogenase (GADPH) and B-tubulin, causing polymerization of microtubules and actin filaments leading to arrest the cell cycle at G0/G1 phase, thus exhibiting higher antiproliferative activity.

The anticancer potential of C-PC extracted from Spirulina platensis is very well known. Phycocyanin induces apoptosis in the existing as well as proliferating cancer cells. Various mechanisms of its anticancer activity are clear from Table 3.
TABLE 3: VARIOUS MECHANISMS OF ANTITUMOR ACTIVITY OF C-PC ISOLATED FROM SPIRULINA PLATENSISS

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Mechanism</th>
<th>Study</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Interference of DNA synthesis in the tumor cells</td>
<td>Wang et al</td>
<td>2001</td>
</tr>
<tr>
<td>2</td>
<td>Activation of caspase-dependent programmed cell death pathways (apoptosis)</td>
<td>Pardhasaradhi et al</td>
<td>2003</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Li et al</td>
<td>2006</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Roy et al</td>
<td>2007</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Li et al</td>
<td>2007</td>
</tr>
<tr>
<td>3</td>
<td>Inhibition of tumor cell growth by membrane destruction, leading to increased leakage of cell constituent</td>
<td>Abd El-Baky</td>
<td>2003</td>
</tr>
<tr>
<td>4</td>
<td>Inhibition of tumor cell growth by stimulation of expression level of the protooncogene c-myc</td>
<td>Liu et al</td>
<td>2000</td>
</tr>
<tr>
<td>5</td>
<td>Improvement of host immune functions</td>
<td>Hayashi et al</td>
<td>2006</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Li et al</td>
<td>2010</td>
</tr>
</tbody>
</table>

Gantar et al demonstrated that in contrast to the C-PC of Spirulina platensis, the C-PC from the cyanobacterium Limnothrix species exhibited low activity. Previous studies have reported that phycocyanin from Spirulina platensis inhibited the growth of human hepatocellular carcinoma cell line SMMC-7721, human rectal cancer cell line HR8348, and human leukemia HL-60, as well as K562 and U937 cell line. Phycocyanin from Porphyra haitanensis was also reported to inhibit the growth of HL-60 cells. Phycocyanin inhibits the growth of Ehrlich Ascites Carcinoma Cells (EACC) in a dose dependent manner, by a pathway other than the apoptosis, by membrane destruction, which led to increase in the leakage of cell constituent and increase in GST and LDH enzyme activities. Spirulina maxima and its protein extract mainly C-phycocyanin provided moderate genotoxic protection (~30%) against hydroxyurea and some protection against the hydroxyurea induced cytotoxicity in mice.

C-phycocyanin attenuates cisplatin-induced nephrotoxicity in mice. Hence, Phycocyanin have antitumor activity and may be used as a chemopreventive agent.

Effect on Prostate Cancer:
Gantar et al reported that when only 10% of standard dose of topotecan was combined with C-PC, the prostate cancer cells (LNCaP) were killed at a higher rate than when topotecan was used alone at full dose. C-PC induce apoptosis through generation of ROS and activation of caspase-9 and caspase-8.

Melanogenesis Inhibitor:
C-phycocyanin is a potential melanogenesis inhibitor. C-phycocyanin inhibits melanin biosynthesis by dual mechanisms; the promoted MITF protein degradation via MAPK/ERK signaling pathway upregulation, and the suppressed activation of CREB via the p38 MAPK pathway down-regulation as is clear from Table 4.

<table>
<thead>
<tr>
<th>Negative impact</th>
<th>Positive impact</th>
</tr>
</thead>
<tbody>
<tr>
<td>-C-phycocyanin may also exert its negative impact on p38 phosphorylation</td>
<td>-C-phycocyanin elevates the cellular abundance of cAMP</td>
</tr>
<tr>
<td>-to restrict activation of the CREB</td>
<td>-which triggers the activation of down-stream MAPK/ERK pathway</td>
</tr>
<tr>
<td>-resulting in restricted MITF gene expression</td>
<td>-leading to the reduction of MITF proteins</td>
</tr>
<tr>
<td></td>
<td>-activation of ERK1/2 resulted in the phosphorylation of MITF at S73</td>
</tr>
<tr>
<td></td>
<td>-induced the subsequent ubiquitin-dependent proteasomal degradation of MITF</td>
</tr>
</tbody>
</table>

The structure resemblance of C-phycocyanin constituents to MAPK pathway modulators (like SB203580 and bilirubin), account for its antimelanogenic effect. SB203580 [4-(4'-fluorophenyl)-2-(4'-methylsulfinylphenyl)-5-(4'-pyridyl) imidazole] acts as a competitive inhibitor of ATP binding of MAP kinase homologues p38a, p38b and p38b2, and blocks α-MSH induced melanogenesis in B16 cells. The prosthetic group of C-phycocyanin, phycocyanobilin might possess similar pyridinyl imidazole structural features to that of SB203580, so, sharing comparable inhibitory mechanisms. In constrast, a structurally related molecule of phycocyanobilin, bilirubin, have an anticancer activity through the MAPK/ERK pathway activation.
at the cytoplasm.\textsuperscript{37} Phycocyanobilin, could function as either or both an ERK activator and a p38 MAP kinase inhibitor to regulate melanin synthesis.\textsuperscript{37}

**Hematological Role:**

In chronic leukemias, including chronic myeloid leukemia (CML), Philadelphia negative myeloproliferative neoplasms (MPNs), and chronic lymphocytic leukemia (CLL), evidence of defects in the regulation of cellular signaling pathways has been reported. Currently, treatment of a CML patient is usually based on a potent protein-tyrosine kinase inhibitor such as imatinib. Imatinib inhibits the kinase activity of the BCR-ABL oncoprotein, thus hindering cell proliferation. Although imatinib is an effective medication, drug resistance is a problem. Therefore, an alternative medicine is needed.\textsuperscript{5} Phycocyanin is an antioxidant protector of human erythrocytes against peroxyl radicals.\textsuperscript{78}

The anticancer effects of C-PC appear to mediated by various mechanisms such as an increase in the proapoptotic Fas protein, down-regulation of Bcl-2, and selective inhibition of COX2. The pathway through which C-PC exerts its activities will enable its application as a therapeutic agent for MPNs.\textsuperscript{5} Tantirapan et al found that C-PC at a micromolar level exhibited a cytotoxic effect on human erythromyeloid leukemia cell line (K562 cells).\textsuperscript{5}

Subhashini et al demonstrated a decrease to approximately 65% of the control growth of K562 cells treated with C-PC. Decrease in the cell growth is a result of apoptosis via downregulation of anti-apoptotic Bcl-2, release of cytochrome c into the cytosol, and cleavage of poly(ADP) ribose polymerase (PARP). C-PC induce apoptosis and inhibit proliferation of cancer cells by altering signal transduction related to both apoptosis enhancement and terminated proliferation in cancer cells, possibly through the Ras/Raf/Mek/ERK, JAK/STAT, MAPK and PI3K/AKT pathways in K652 cells.\textsuperscript{5}

**Hepatocellular Carcinoma (HCC):**

Roy et al demonstrated a 50% decrease in proliferation of doxorubicin sensitive (S-HepG2) and doxorubicin resistant (R-HepG2) HCC cell lines with phycocyanin. C-PC also enhanced the sensitivity of R-hepG2 HCC cells to doxorubicin. They showed downregulation of the anti-apoptotic protein Bcl-2 and upregulation of the pro-apoptotic Bax protein in the R-HepG2 cells.\textsuperscript{79} Nishanth et al reported a significant down regulation of MDR1 (multidrug resistance-1) expression in C-PC treated HepG2 cells through cyclooxygenase-2 (COX-2) and reactive oxygen species mediated pathways. In a concentration dependent manner, C-PC increased the doxorubicin accumulation in HepG2 cells and enhanced cells sensitivity to doxorubicin by 5 folds. Further studies reveal the involvement of AP-1 and NF-kB in the C-PC induced down regulation of MDR1. The inactivation of the signal transduction pathways involving ERK, Akt, p38 and JNK by C-PC was also observed.\textsuperscript{80}

Ou et al produced hepatoprotective activity of C-phycocyanin against carbon tetrachloride-induced hepatocyte damage in vivo and in vitro. Mechanisms involved through C-PC’s scavenging ability and ability to block inflammatory infiltrate through its anti-inflammatory activities by inhibiting HGF and TGF-β1 expression.\textsuperscript{81} Phycocyanin being COX-2 inhibitor, significantly inhibits liver microsomal lipid peroxidation hence protecting the liver by preventing oxidative stress in hepatocytes.\textsuperscript{82} Phycocyanin inhibits microsomal lipid peroxidation induced by Fe+2 – ascorbic acid or the free radical initiator 2, 2’ azobis (2-amidinopropane) hydrochloride (AAPH).\textsuperscript{83} C-PC extracted from Porphyra yezoensis could develop to new photosensitizers for cancer photodynamic therapy.\textsuperscript{84}

**Colon Carcinoma:**

Lu et al reported that the recombinant α-subunit of C-phycocyanin (CpcA) inhibited the growth of human colon carcinoma COLO 205 cells. The apoptotic process was associated with the Bax/Bcl-2 ratio up-regulation, mitochondrial membrane depolarization, cytochrome c release, and caspase-9 activation. It was proven that CpcA induced the death of COLO 205 cells through intrinsic apoptotic pathway.\textsuperscript{85}

**Breast Cancer:**

Phycocyanin is an ideal photosensitizer which accumulates in breast cancer tissue and attracts He-Ne laser to target at tumor tissues. Being natural
and non-toxic, phycocyanin is a good substitute to highly toxic photosensitizers or chemotherapeutic drugs. It also causes inhibition of MCF-7 cell proliferation and morphological changes like chromatin condensation, blebs formation and loss of microvilli. C-PC-mediated photodynamic therapy activates immune system, induce pro-apoptotic Fas genes activation, cause apoptotis of cancer cells and down-regulates anti-apoptotic protein expression such as P53, Bcl-2, NF-kB and CD44 mRNA. Hence, Phycocyanin could be the new potential anticancer drug for therapy of Human Breast Cancer. 

Cervix Cancer:
Treatment of Human cervical cancer cell line (HeLa) cells with Phycocyanin increases hypodiploid cells population and DNA fragmentation. Phycocyanin reduces antiapoptotic proteins Bcl-2 level and promote death receptor genes expression like Fas/FasL and ICAM. Caspases play a central role in all apoptotic pathways and a higher level of caspases are seen in phycocyanin treated cells. Hence, Phycocyanin might be the new potential anticancer agent for Cervical Cancer therapy.

Phycocyanin and Laser Therapy:
In this photochemical method, Phycocyanin is injected into a patient suffering from atherosclerosis or cancer. After being injected, phycocyanin is taken up selectively into the atherosclerotic plaques or the cancer cells. Destruction of the atherosclerotic plaques or the cancer cells occurs on subsequent irradiation. Phycocyanin has several advantages over prior art chemicals used for similar purposes. First, it is only marginally sensitive to ultraviolet part of the spectrum; consequently the patients can be irradiated without taking concern that they will be sensitized to subsequent sunlight exposure. Second, phycocyanin is taken up selectively into the atherosclerotic plaques, with little or no uptake by the surrounding normal cells. This ensures that upon subsequent irradiation, atherosclerotic plaques are selectively destroyed with little or no damage to surrounding cells or tissue.

Effect on Pancreas:

Li et al demonstrated that cleavage of poly (ADP-ribose) polymerase (PARP) and activation of caspase-3 was blocked by Phycocyanin in hIAPP-treated cells. Also, PC significantly prevented the hIAPP-induced overproduction of malondialdehyde (MDA) and intracellular ROS, as well as changes in activities of glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) enzymes. Furthermore, hIAPP triggered mitogen-activated protein kinases (MAPKs) activation, but these effects were effectively suppressed by PC. So, the study reported that PC protects INS-1E pancreatic beta cells of rat insulinoma against hIAPP-induced apoptotic cell death through attenuating oxidative stress and modulating c-Jun N-terminal kinase (JNK) and p38 pathways. This is not proved in humans till now. PC may have a potential to treat type-2 diabetes mellitus as it enhances insulin sensitivity, regulates glucolipide metabolism and ameliorates insulin resistance of peripheral target tissues.

Ischemia Reperfusion Injury:
C-phycocyanin could be used for treating ischemia-reperfusion injury through the activation of ERK pathway and suppression of p38 MAPK pathway. Phycocyanin may be used to treat ischemic stroke as phycocyanobilin has an effective influence on major inflammatory mediators of acute cerebral hypoperfusion.

Cardiovascular Disease (CVD) and Non-Alcoholic Fatty Liver Disease (NAFLD):
 Chronic diseases, such as CVD and NAFLD, are highly related to impaired lipid metabolism, oxidative stress, and inflammation. Phycocyanin provides multiple health-promoting properties: inhibition of inflammation via decreased nuclear factor kappa B (NF-kB) activity; lowering plasma lipid concentrations by decreasing intestinal cholesterol absorption and hepatic lipogenesis; and prevention of oxidative stress by blocking lipid peroxidation and increasing free radical scavenging. In conclusion, Phycocyanin can be consumed as a dietary supplement or a food component to obtain health benefits against CVD and NAFLD.
The anti-inflammatory, antioxidant and immune-modulatory properties contribute to the neuroprotective effects of Phycocyanin. Either the therapeutic or prophylactic application of Phycocyanin is able to significantly reduce the infarct volume, and also protect hippocampal neurons from death, induced by cerebral ischemia or reperfusion injury. Phycocyanin is platelet aggregation inhibitor with a potential to hamper arterial thromboembolism.\textsuperscript{94}

**Nephroprotective Effects:**
Phycocyanin protects the renal cell integrity by stabilizing lipid peroxidation and protecting against oxalate induced nephro injury. Lipid peroxidation produce aldehydes like malondialdehyde (MDA) that are extremely active and can diffuse within or even escape from the cell and attack targets far from the site of the original free radical initiated event, resulting in cell damage and therefore act as ‘cytotoxic second messengers’. Phycocyanin pre-treatment decreased the lipid peroxidation and reversed the effects of oxalate on oxidative stress parameters by interacting with hydroxyl radical and by rebalancing the GSH content, catalase and G6PD activity in oxalate treated animals.\textsuperscript{95}

**Acute Lung Injury:**
Acute lung injury (ALI) is characterized by damage to the epithelial and endothelial cells in lungs, mediated by several pro-inflammatory mediators and finally impairs respiratory function. Phycocyanin exhibits its anti-inflammatory activity by inhibiting inducible nitric oxide synthase (iNOS) expression and NO production possibly by suppressing nuclear transcription factor-kB (NF-kB) activation, a key transcription factor promoting proinflammatory gene expression. Leung et al reported that posttreatment of ALI model with C-PC significantly reduces the tissue permeability, and protein concentration in bronchoalveolar lavage fluid (BALF) and improves pulmonary histological alterations.\textsuperscript{96}

**Wound Healing:**
Phycocyanin directly enhances wound repair by its anti-oxidant property and scavenging destructive free radicals mechanism. Secondly, stimulation of keratinocyte is one mechanism by which phycocyanin might enhance wound repair process.\textsuperscript{97}

**Side Effects:** Phycocyanin is usually non-toxic, but may cause liver damage, stomach pain, nausea, vomiting, weakness, thirst, rapid heartbeat, shock, and death. Phycocyanin may cause systemic anaphylaxis, urticaria, labial edema, asthma, diarrhea and diffuse erythema.\textsuperscript{98}

**CONCLUSION:** Phycocyanin is a natural product from cyanobacteria and is a rich source of antioxidants. In view of current practice, antioxidant properties of phycocyanin have been investigated for their anti-inflammatory, anti-proliferative and anti-cancer effects. The results revealed for the first time that the C-phycocyanin activities and its antitumor actions such as in leukemia, colon cancer, pancreatic cancer etc. could be a promising natural antitumor agent with a potential for future pharmacological and medical applications. In the future, these marine algae-derived materials or compounds will be used more often in pre-clinical studies for drug discovery. In our review article, like other anti-cancer agents, phycocyanin being a natural product, should be more acceptable as an anticancer compound.

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**REFERENCES:**
5. Tantirapan P, Suwanwong Y: Anti-proliferative effects of C-phycocyanin on a human leukemic cell line and induction of...


76. Singh SK, Sarkar C, Mallick S, Saha B, Bera R, Bhadra R: Human placential lipid induces malangenesis through p38...

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