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New Scientific Developments in the Health Benefits of *Spirulina (Arthrospira)*: Phycocyanin and its Potential Health Benefits

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This paper reviews the available published literature on the antioxidant, anti-inflammatory, and COX-2 inhibition properties of phycocyanin from *Spirulina*. The potential application of for the prevention and mitigation of such radical-induced chronic diseases like cancer and heart disease, and age-related degenerative diseases like Alzheimer's disease, Parkinson's disease, diabetes and other conditions are discussed based on the available evidence.

Key words : *Spirulina (Arthrospira)*, phycocyanin

INTRODUCTION

Spirulina (Arthrospira) has been the subject of food, feed and therapeutic interest for almost three decades. It has a long history of use as food having been the food of the Aztecs in Mexico and being used as food by the Kanebu tribe in the Lake Chad area of the Republic of Chad. *Spirulina* has also been commercialized as food and dietary supplement for the last 30 years.[1)]

Recently the interest in *Spirulina* focuses on its potential as a natural therapeutic supplement in the management of health. Some important components of *Spirulina* are being identified that point to some significant potential health benefits. Phycocyanin is one such component that is the subject of recent research.

In the first ever published review of the health benefits of *Spirulina* Belay et al.[2)] called the attention of researchers to look into some aspects of the health benefits of *Spirulina*. Since then numerous studies have been established that showed the great potential that these algae have in the maintenance of various aspects of health. These studies were the subject of a presentation at the International Symposium on *Spirulina* and Health held in Korea.[3)] Since then Belay (2002) has provided an extensive review of the published literature on the health benefits of *Spirulina*.[4)]

It is not the intention of this paper to give a detailed presentation of the many studies on the health benefits of *Spirulina*. This paper will focus more on recent scientific studies on the health benefits of phycocyanin, an important component of *Spirulina*.

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Antioxidant Effects of Phycocyanin from Spirulina (Arthrospira)

Oxygen that is so vital to our metabolism and energy production can also be dangerous at times because such energy releasing reactions also result in the formation of free radicals. In addition, environmental factors like poor diet, stress, smoking and toxic chemicals also contribute to the formation of these free radicals. Free radicals are unstable molecules that can damage cell structures and lead to many degenerative diseases. It is now well established that diseases like cancer, heart disease, Alzheimer's disease, Parkinson's disease, diabetes, cataracts, arthritis, multiple sclerosis are either caused by free radical damage or aggravated by them.[5)]

In a healthy body these free radicals and reactive oxygen species are balanced by our network of antioxidants derived from our diets or endogenously. However such free radical-generating reactions take place in our body at very high frequencies. For example, it has been estimated that the DNA in each human cell receives something like 10,000 oxidative hits a day. Poor diet, stress and other environmental factors may create imbalance in the body's antioxidant balance and result in undesirable consequences. The most frequent damage caused by free radicals is lipid peroxidation which generates a cascade of events resulting in many health problems. Free radicals also attack lysosomes which produce essential enzymes necessary for body defense, in addition to changing protein structure and function.

The relationship between antioxidant intake and incidence of chronic diseases like cancer, cardiovascular disease and premature aging is now well established through several epidemiological, intervention and clinical studies. In particular there is a strong association between the intake of fruits and vegetables and the incidence of cancer. This

has in fact led the National Cancer Institute to initiate the 5-A-Day Program in 1991 recommending at least 5 servings of fruits and vegetables a day.[6] Surveys show that only 25% of adult Americans take the recommended level. It is often said that the fast style of life does not allow for the fulfillment of these requirements. This may explain the high increase in the demand of anti-oxidant nutraceuticals which provide an easy way to supplement the diet. It is in this context that the following review on the antioxidant properties of phycocyanin is presented.

Many in vitro and some animal studies using different systems of generating oxygen free radicals and reactive oxygen species have shown that *Spirulina*, and in particular its phycocyanin extract shows promise as a natural antioxidant of significant health benefit.

Alkoxyl, Hydroxyl and Peroxyl Radical Scavenging Activities of Phycocyanin

The antioxidant effect of phycocyanin has been the subject of a recent extensive review.[7] Romay et al.[8] demonstrated the antioxidant effect of *Spirulina* phycocyanin using a chemiluminescence (CL) assay. In their system, the inhibition of alkoxyl radical was studied by measuring the inhibition of CL produced by the reaction of tert-butyl hydroperoxide with ferrous ions in the presence of luminol. Trolox was used as a control to compare the antioxidant activity. The results showed that c-phycocyanin from *Spirulina* inhibited CL with an IC_{50} of 76 μg/mL. The IC_{50} of Trolox was 0.038 μg/mL.[8]

Romay's group also studied the hydroxyl radical scavenging activity of phycocyanin by measuring the inhibition of CL produced by the Fenton reaction with luminol. Phycocyanin inhibited CL in a dose-dependent manner with an IC_{50} of 0.91 mg/mL compared to 0.125 mg/mL for DMSO which was used as control in this case.[8] The hydroxyl radical scavenging properties of phycocyanin were also assessed in another model of inhibition of damage to 2-deoxyribose measured as formation of thiobarbituric acid reactive substances (TBARS). Phycocyanin inhibited deoxyribose damage in a concentration-dependent manner with an IC_{50} of 0.86 mg/mL and a second order rate constant comparable to some known NSAIDs such as indomethacin and ibuprofen.[8] Similar results were obtained by Bhat & Madyastha[9] who demonstrated that it was the bilin chromophore that was responsible for the hydroxyl radical scavenging property of phycocyanin. Bhat & Madyastha[9] also found that phycocyanin protected deoxyribose when the deoxyribose assay was done in the absence of EDTA suggesting that phycocyanin chelates with iron and protects the target molecule, deoxyribose. The IC_{50} of phycocyanin inhibition of deoxyribose degradation was 28 μM in the presence of EDTA and only 13 μM in the absence of EDTA.[9]

Using the progressive decrease in the visible absorbance of phycocyanin with peroxyl radicals generated by the thermolysis of 2,2 Azo-bis(2-amidinopropane) hydrochloride (AAPH), Lissi et al[10] were able to calculate the bilin groups destroyed per radical that interacts with the protein. Micromolar concentrations of phycocyanin could reduce the steady state concentration of the peroxyl radical by 50%. Bhat & Madyastha.[9] did a similar study using the same method as above and a crocin bleaching assay to measure the peroxyl radical scavenging activity of phycocyanin. Co-incubation of phycocyanin with AAPH (10 mM) at 37℃ resulted in a significant decrease in the absorption at 618 nm (60% decrease). The decrease in absorption was accompanied by the disappearance of color. Moreover, competition kinetics of crocin bleaching revealed phycocyanin as being a potent peroxyl radical scavenger with an IC_{50} of 5.0 μM as compared with an IC_{50} of 1.9 μM for uric acid, a known peroxyl radical scavenger. Both native phycocyanin and its reduced form were found to scavenge peroxyl radicals.[9]

Effects of Phycocyanin on Lipid Peroxidation

Using human erythrocytes and treating them with Fe^{+2} and ascorbic acid mixture Manoj et al.[11] measured lipid peroxidation using the concentration of TBARS as an index of lipid peroxidation. Both the alcoholic and water extracts of *Spirulina* showed significant inhibition of lipid peroxidation. The alcohol extract inhibited lipid peroxidation significantly more (65% inhibition) than the well-known antioxidants like α-tocopherol (35%), BHA (45%) and β-carotene (48%). The water extract of *Spirulina* also showed more radical scavenging activity (76%) compared to gallic acid (54%) and chlorogenic acid (56%).[11] In their study the alcohol extract may be exhibiting antioxidant activities of mixed carotenoids found in *Spirulina* while the effect of the water extract may be due to the phycocyanin component as found in many other studies. In a similar assay, Romay et al.[8] also showed that the addition of phycocyanin (200-540 μM) resulted in an inhibition of liver microsomal peroxidation induced by Fe^{+2}-ascorbic acid with an IC_{50} of 12 mg/mL. Both the rate and the final degree of lipid peroxidation were reduced by adding c-phycocyanin. Hirata et al..[12] found that the formation of linoleic acid hydropereoxides initiated by AAPH was inhibited significantly in the presence of phycocyanobilin and other antioxidants. Quercetin showed the strongest antioxidant activity followed by caffeic acid, chlorogenic acid and catechin. Bhat & Madyastha[9] used an in vivo model of lipid peroxidation where they induced lipid peroxidation by the intraperitoneal administration of carbon tetrachloride (CCl_4) in rats at a dose of 0.6 mL/kg. The effect of phycocyanin on CCl_4-induced lipid peroxidation was studied by feeding rats with phycocyanin (50-200 mg/kg body weight) dissolved in water 3 hours prior to the administration of CCl_4. The extent of hepatic lipid peroxidation was studied by measuring the production of

malonaldehyde (MDA) in liver homogenates. The liver MDA level was nearly 5 times higher in CCl_4- treated rats than the control rats or rats treated with the phycocyanin and CCl_4. In vitro studies on the effect of phycocyanin on AAPH-initiated peroxyl radical lipid peroxidation showed a concentration-dependent inhibition of lipid peroxidation with an IC_{50} of 11.35 μM. At 200 μM concentration, phycocyanin inhibited close to 95% of lipid peroxidation.[9]

Phycocyanin was also shown to protect human erythrocytes from lysis by peroxyl radicals in the same way as trolox and ascorbic acid, well-known antioxidants. The IC_{50} value of phycocyanin (12-75 μM) in this model was found to be almost sixteen times that of trolox and about twenty times more than ascorbic acid.[13]

Effects of Phycocyanin on Peroxynitrite and Hypochlorite Reactions

Scavenging of ONOO(-) by phycocyanin and its chromophore, phycocyanobilin was established by studying their interaction with ONOO(-) and quantifying the activity by using competition kinetics of pyrogallol red bleaching assay.[14] The relative antioxidant ratio and IC_{50} value clearly showed that phycocyanin is a more efficient ONOO(-) scavenger than phycocyanobilin. They also found that phycocyanobillin significantly inhibited the ONOO(-) -mediated single-strand breaks in supercoiled plasmid DNA in a dose-dependent manner with an IC_{50} value of 2.9 +/- 0.6 μM thus protecting the DNA from oxidative insult.[14]

The kinetics of phycocyanin reaction with hypochlorite was recently elucidate by Romay et al..[15] The antioxidant activity of phycocyanin was studied by following the decrease in absorbance at 620 nm with time. It was found that the apoprotein component was important in the antioxidant effect. However the reaction rate was found to be too slow to have any significant antioxidant or anti-inflammatory effect.

Anti-inflammatory Effects of Phycocyanin from *Spirulina*

The anti-inflammatory property of phycocyanin has been studied in various models of inflammation by several people. These studies are summarized below.

Inhibition of Paw and Ear Oedema Induced by Various Substances

In what appears to be the first study on anti-inflammatory properties of phycocyanin, Romay et al.[8] demonstrated that phycocyanin (at doses of 100 and 200 mg/kg p.o.) significantly reduced the paw oedema induced by peroxide in a dose-dependent manner. The anti-inflammatory effect was ascribed partly to the scavenging of hydroxyl radical by phycocyanin. The hydroxyl radicals were generated by the injection of glucose oxidase into the mouse paw and its reaction with endogenous glucose to first form hydrogen peroxide. Further studies by the same group[16] revealed the anti-inflammatory effect of phycocyanin in some animal models of inflammation. Phycocyanin (50-200 mg/kg, P.O.) supplied one hour before arachidonic acid (AA) application, reduced significantly and in a dose-dependent manner the ear oedema induced by the topical application of AA in the ears of mice. The effective dose that results in 50% reduction (ED_{50}) was 66.1 mg/kg. In their carageenan- induced rat paw oedema study, subplanar injection of carageenan resulted in an increase in paw thickness. The administration of phycocyanin one hour before the injection of carageenan inhibited paw oedema as measured by paw thickness in a dose-dependent manner. Indomethacin (10 mg/kg, P.O.) also resulted in the inhibition of paw oedema. When compared with indomethacin, a standard anti-inflammatory drug, phycocyanin showed a weaker activity (50-300 mg/kg, p.o.) compared to 3-10 mg/kg p.o. for the former. However, the LD_{50} of indomethacin was 12mg/kg in rats and 50 mg/kg in mice, p.o. and induces many side effects in patients under treatment. The LD_{50} of phycocyanin in rats and mice was greater than 3 g/kg, P.O.. Phycocyanin was also shown to inhibit TPA-induced mouse ear oedema and cotton pellet induced granuloma in rats.[16] Romay et al.[17] also found that ear oedema induced by AA and leukotriene B_4 (LTB_4) concentration were reduced significantly by oral administration of phycocyanin (100 and 200 mg/kg, p.o.) one hour prior to AA application. The effect on LTB_4 is postulated to be due to the reduction of enzymatic and non-enzymatic lipid peroxidation via AA metabolism as well as inhibition of the lipoxygenase enzyme directly. This contention was later partly proven in another study.[18] In this study phycocyanin (50-200 mg/kg, p.o.) reduced in a dose-dependent manner PGE-2 levels in mouse ear treated with AA and moderately reduced PLA_2 levels in TPA-induced mouse ear inflammation test showing the possible involvement of metabolites of AA via cyclooxygenase (CO) and lipoxygenase (LO) pathways.

Inhibition of Aceitic Acid-induced Colitis in Rats

The anti-inflammatory effect of c-phycocyanin extract was studied in acetic acid-induced colitis in rats.[19] Colitis was induced by enema of 1 mL of 4% acetic acid per rat as a model of inflammatory bowel syndrome (IBD). The latter is believed to be caused by metabolites of arachidonic acid such as leukotrienes and prostaglandins and reactive oxygen species such as superoxides, hydrogen peroxide, hydroxyl radicals and HOCl. Since phycocyanin has been shown to scavenge oxygen radicals and interfere with arachidonic acid metabolites, the potential for the treatment of IBD was evaluated in this study. The study involved the administration of phycocyanin (150, 200, 300 mg/kg p.o.) 30 minutes before induction of enema with acetic acid,

and the determination of myeloperoxidase (MPO) activity. Histopathological and ultrastructural changes in the colonic tissue were also observed. Acetic acid-induced colitis resulted in significant increases in colonic MPO content of normal control rats. However this effect was reduced significantly in rats which were pre-treated with phycocyanin prior to the induction of colitis. The effect was greater at the higher doses of 200 and 300 mg/kg, p.o.. The effect was believed to be due to reduction of neutrophil infiltration in colonic tissue. Phycocyanin at 200 mg/kg, P.O. showed a comparable degree of inhibition of colitis as 5-aminosalicylic acid (5-ASA) at 200 mg/kg. 5-ASA has been shown to have a beneficial effect in IBD. Histological observations showed that rats that were not treated with acetic acid had a normal mucosa in contrast to the treated group that were observed to have mucosal haemorrhage, severe inflammatory cell infiltration, submucosal oedema and focal ulceration. In the rats pre-treated with phycocyanin, there was only slight submucosal oedema and mild inflammatory cell infiltration. Ultrastructural observations also revealed loss of microvilli in the colonic tissue or rates treated with acetic acid. This effect was slightly reversed by phycocyanin at 300 mg/kg, P.O..[19]

Inhibition of Zymosan-induced Arthritis in Mice

Although the exact cause of rheumatoid arthritis (RA) is not known, it is believed that several inflammatory mediators like prostaglandins and leukotrienes, tumor necrosis factor (TNF), interlukinn-1 (IL-1) and IL-6 and reactive oxygen species are involved. RA is characterized by joint inflammation and eventually cartilage destruction. Earlier findings about the antioxidant effects of phycocyanin in several in vitro and animal models led Remirez et al.[20] to study the effect of phycocyanin on zymosan-induced arthritis model in mice. The study involved the administration of phycocyanin (25, 50, and 100 mg/kg, p.o.) to mice four days after intraarticular injection of zymosan, and continuing feeding with phycocyanin for 8 days at which time the mice were killed and the synovial fluid measured for β-glucuronidase. Histopathological and ultrastructural examinations were made of the knee joint. Phycocyanin significantly reduced the level of β-glucuronidase that had been increased by zymosan. Almost complete inhibition of enzymatic activity was attained at 100 mg/kg p.o. level of phycocyanin. This was comparable to the effect of triamcinolone, the reference drug used in these studies. The histopathological studies also showed inhibition of cellular infiltration and reduction of synovial hyperplasia and synovitis.[20]

According to these authors, agents of arthritis like zymosan are known to activate complement by alternative and classical pathways and induce secretion of macrophage lysosomal enzymes. Moreover, zymosan is a powerful releaser of AA metabolites while reactive oxygen species such as hydroxyl radicals, hydrogen peroxide and HOCl are known to be involved in rheumatic arthritis. TNFα and other inflammatory cytokines are also known mediators of zymosan-induced arthritis. Taking all this into consideration they concluded that the antiarthritic effect of phycocyanin found in their study was probably due to its antioxidant and ROS scavenging activities possibly also due to inhibition of production of cytokines like TNFα.[20] In this regard Romay et al.[21] have found that phycocyanin (50-300 mg/kg p.o.), administered 1 hour before treatment of mice with lipopolysaccharide (LPS), reduced the level of TNFα in serum dose-dependently. Serum nitrite levels increased significantly after 18 hours of LPS (30 mg/kg i.p) but were reduced significantly by pretreatment with phycocyanin (100-300 mg/kg p.o.).

Hepatoprotective Effects

Vadiraja et al.[22] studied the effect of c-phycocyanin from *spirulina* on carbon tetrachloride and R-(+)-pulegone-induced hepatotoxicity in rats. In this study a single dose (200 mg/kg) of phycocyanin was administered intraperitoneally to rats one or three hours prior to R-(+)-pulegone (250 mg/kg) or carbon tetrachloride (0.6 mL/kg) challenge. Phycocyanin significantly reduced the hepatotoxicity caused by these chemicals. Both chemicals are believed to cause hepatotoxicity due to the formation of free radicals. The hepatoprotective effect of phycocyanin was therefore attributed to the inhibition of reactions involved in the formation of reactive metabolites and possibly due to its radical scavenging activity. Torres-Duran et al.[23] have also found a similar hepatoprotective effect against CCl_4-induced hepatotoxicities in feeding experiments of rats with an oil extract of *spirulina* or its defatted fraction. They showed that the values of the liver microsomal thiobarbituric acid-reactive substances were lower in the *Spirulina* group than in the control group. These results support the potential hepatoprotective role of *Spirulina* probably due to its antioxidant effect. Recently, Bhat and Madayastha[9] reported that c-phycocyanin from *Spirulina* effectively inhibited CCl_4-induced lipid peroxidation in rat liver *in vivo*. The inhibition by both native and reduced phycocyanin was concentration-dependent with an IC_{50} of 11.35 and 12.7 μM, respectively. Their studies have shown unequivocally that phycocyanin is a potent peroxyl radical scavenger with a rate constant ratio of 1.54 compared to 3.5 for uric acid (a known peroxyl radical scavenger).

According to Upasani et al.[24] concomitant administration of lead (100 ppm) and *Spirulina* (1500 mg/kg) in food for 30 days resulted in a significant reduction of lipid peroxidative products like malonaldehyde, conjugated dienes and hydroperoxides in the liver, and kidney of mice treated with *Spirulina* compared to untreated controls. This was also shown to be true for vitamin E and vitamin C suggesting that the antioxidant function of *Spirulina* in the

liver and kidneys of these animals.

More recently, Remirez et al.[25] did a study on the effect of c-phycocyanin on some hepatocellular parameters related to liver oxidative stress and Kupffer cell functioning. Kupffer cells are liver macrophages involved in immunomodulation, phgaocytosis and biochemical attack and can induce cytotoxicities and inflammation when they are over-stimulated. Colloidal carbon phagocytosis (as a measure of Kupffer cell phagocytosis) and carbon-induced respiratory burst activity, and sinusoidal lactate dehydrogenase (LDH) release were studied in isolated perfused mouse liver. The study also looked at the influence of c-phycocyanin on TNFα and nitrite levels in serum and liver nitric oxide synthase (NOS) activity in rats subjected to 3,3',5-triiodothyronine (T3) administration as a measure of hepatic oxidative stress and Kupffer cell activity. The results show that phycocyanin inhibited carbon phagocytosis and carbon-induced oxygen uptake (IC_{50} = 0.2 mg/mL) by perfused livers. At 0.25 mg/mL, phycocyanin elicited a 52% reduction in the carbon-induced sinusoidal release of LDH showing the integrity of the plasma membrane of hepatocytes and other cells in the liver and hence the hepatoprotection by phycocyanin. Furthermore, thyroid calorigenesis induced an 82-fold increase in serum levels of TNFα that was reduced significantly by pretreatment with phycocyanin, α-tocopherol, and by the Kupffer cell inactivator gadolinium chloride. Phycocyanin also suppressed the T-3-induced increases in serum nitrite levels (234%) and hepatic NOS (75%). According to these authors, the inhibition of Kupffer cell-dependent respiratory bust activity may result from the observed reduction in particle phagocytosis and/or to a direct scavenging of free radicals produced in the process.

Neuroprotective Effects

In an elaborate study involving neurobehavioral changes including "wet dog shakes", tremors and seizures and body weight, and indirect markers of neuronal damage as measured by equilibrium binding assays to peripheral benzodiazepine receptor (PBR) and heat shock protein 27kD (hsp27kD) in the hippocampi of rats, Rimbau et al.[26] have shown that oral administration of c-phycocyanin (100 mg/kg) prevents kainic acid-induced behavioral and glial activity in the rat hippocampus suggesting a corresponding protective effect on neurons. The study showed that phycocyanin reduced experimental status epilepticus suggesting a possible therapeutic intervention in the treatment of some forms of epilepsy. According to the authors, KA-triggered excitotoxicities resulted in the production of reactive oxygen species (ROS). It is therefore postulated that the protective effect of phycocyanin in neuronal damage may be due to its free radical scavenging and antioxidant properties. An interesting aspect of this study is the finding that oral administration of phycocyanin exerts its effect in the hippocampus crossing the hematoencephalic barrier. According to the authors, these findings and the virtual lack of toxicity of phycocyanin suggest that this phytochemical could be used in the treatment of neurodegenerative diseases such as Alzheimer's and Parkinson's diseases brought on by oxidative stress-induced neuronal injury.[26] More recently, Rimbau et al.[27] tested the potential cytoprotective role of c-phycocyanin in rat cerebellar granule cell (CGC) cultures. The neuroprotective effect of c-phycocyanin in cultured CGC was evaluated 24 hours after potassium and serum (K/S) deprivation. K/S withdrawal induced 60.3±3.8% neuronal death. Pretreatment of CGC with phycocyanin (3mg/mL 24 hours before K/S deprivation) prevented cell death due to K/S removal to the same level as the control without K/S deprivation. Furthermore, K/S deprivation was found to result in morphological changes to CGC resulting in disruption of neuronal network and changes in cell shape. On the other hand, pretreatment with phycocyanin (3 mg/mL) resulted in a cell morphology similar to that observed in control cultures. Phycocyanin (3 mg/mL) also significantly reduced apoptosis induced in CGC by K/S deprivation. This effect of phycocyanin may be important in the therapeutic use of phycocyanin in such neurodegenerative diseases like Alzheimer's, Huntington's disease and amyotrophic lateral sclerosis all of which could result at least partially from apoptotic loss of neurons.[27] Phycocyanin pretreatment was also found to inhibit the increase in thymidine incorporation into DNA induced by K/S deprivation. Moreover, pretreatment with phycocyanin resulted in a significant decrease in the production of ROS induced by K/S deprivation. This and other conditions led the authors to conclude that the apoptosis by K/S deprivation was probably caused by ROS and that the neuroprotective effect exhibited by phycocyanin could be due to its antioxidant effect.

Gemma et al.[28] examined whether antioxidants and diets supplemented with foods high in oxygen radical absorbance capacity (ORAC) reversed age-related decreases in cerebellar beta-adrenergic receptor function. Aged male Fischer 344 rats were given apple (5 mg dry weight), *Spirulina* (5 mg), or cucumber (5 mg) either in 0.5 mL water by oral gavage or supplied in the rat chow daily for 14 d. Electrophysiologic techniques revealed a significant decrease in beta-adrenergic receptor function in aged control rats. *Spirulina* reversed this effect. Apple (a food with intermediate ORAC) had an intermediate effect on cerebellar beta-adrenergic receptor physiology, and cucumber (low ORAC) had no effect, indicating that the reversal of beta-adrenergic receptor function decreases might be related to the ORAC dose. The mRNA of the proinflammatory cytokines tumor necrosis factor-alpha (TNF-α) and TNF-β was also examined. RNase protection assays revealed increased levels of these cytokines in the

aged cerebellum. *Spirulina* and apple significantly down-regulated this age-related increase in proinflammatory cytokines, whereas cucumber had no effect, suggesting that one mechanism by which these diets work is by modulation of an age-related increase in inflammatory responses. Malondialdehyde (MDA) was measured as a marker of oxidative damage. Apple and *Spirulina* but not cucumber decreased MDA levels in the aged rats. According to Gemma et al.[28] the improved beta-adrenergic receptor function in aged rats induced by diets rich in antioxidants is related to the ORAC dose, and because these diets reduce proinflammatory cytokine levels. The fact that the ORAC method measures water soluble antioxidants may point to phycocyanin as the major component that elicited the neuroprotective effect observed in their study. Cartford et al.[29] reported that 6 weeks of a spinach-enriched diet ameliorated deficits in cerebellar-dependent delay classical eyeblink learning in Fischer 344 rats and reduced the proinflammatory cytokines TNF-α and TNF-β in the cerebelli of eyeblink-trained animals. Though conjectural the same effect would be expected from the former study by the same group[28] using *Spirulina*, where a similar type of cytokine regulation was observed with respect to TNF-α and TNF-β. Spirulina is known to modulate a Th1 type of response in favor of a Th2 type response. Mao et al.[30] studied the effect of *Spirulina* on the secretion of three cytokines from unstimulated and stimulated human peripheral blood mononuclear cells. They found an almost 14-fold increase in interferon (INF-γ) and only a 3 fold increase in interleukin (IL-4) suggesting that *Spirulina* favors a Th1 response. The immunomodulatory property of *Spirulina* has been discussed in detail in an earlier review.[4]

Effects Against Allergic Inflammation

Yang et al.[31] did extensive studies on the effect of orally-administered on anaphylactic reaction. The following is a summary of their findings: 1) *Spirulina* inhibited compound 48/80-induced anaphylactic shock 100% with doses of 0.5 and 1.0 mg/g body weight, 2) *Spirulina* significantly reduced serum histamine levels induced by compound 48/80 in rats, 3) Passive cutaneous anaphylaxis activated by anti-dinitro-phenyl IgE was inhibited to 69%, 4) *Spirulina* dose-dependently inhibited histamine release from rat peritoneal mast cells by compound 48/80, and 5) *Spirulina* had a significant effect on the anti-DNP IgE-induced histamine release or tumor necrosis facto-α production from RPMC. The authors postulate that the effects observed are possibly due to inhibition of anaphylactic degranulation of mast cells by *Spirulina*. Subsequent studies by Kim et al.[32] on the effect of *Spirulina* on mast cell-mediated immediate-type allergic reactions in rats also showed similar results. In this study *Spirulina* dose-dependently inhibited the systemic allergic reaction induced by compound 48/80 in rats. Compound 48/80-induced allergic reaction was inhibited 100% with intraperitoneal doses of 100-1000 μg/g body weight. In rats treated with intraperitoneal dosages at concentrations ranging from 0.01 to 1000 μg/g body weight, serum histamine levels were reduced in a dose-dependent manner. *Spirulina* also dose-dependently inhibited histamine release from rat peritoneal cells activated by compound 48/80 or anti-DNP IgE. *Spirulina* also had a significant inhibitory effect on anti-DNP IgE-induced tumor necrosis factor-α production.[32] More recently Remirez et al.[33] have shown a similar role of histamine in the inhibitory effects of phycocyanin in experimental models of allergic inflammation.

Phycocyanin and the Cyclooxygenase (COX-2) Connection

It is now known that the cylooxygenase (COX) enzyme exists in two forms-COX-1 and COX-2. COX-1 is predominantly constitutive and is responsible for the production of prostaglandins important for responses to circulating hormones and maintenance of gastric mucosal integrity and platelet function. COX-2 is an inducible enzyme for the most part and is responsible for the biosynthesis of inflammatory prostaglandins. Its levels can increase ten to twenty fold through inflammation, particularly in macrophages, monocytes, synoviocytes, chondrocytes, fibroblasts and endothelial cells and in malignant tissues from colorectal cancer, human gastric and breast tumors.

The most frequently prescribed medications for anti-inflammatory diseases like arthritis are non-steroidal anti-inflammatory drugs (NSAIDs) like Aspirin. These drugs work by inhibiting the cyclooxygenase enzyme in a non-selective manner affecting both types of cyclooxygenase enzymes. As a result they affect COX-1 enzyme expression and result in undesirable side effects particularly upper gastrointestinal complications including symptomatic and complicated ulcers. The desire to avoid the side effects of traditional NSAIDs has lead to the development of a new class of drugs called selective cyclooxygenase-2 (COX-2) inhibitors or "super aspirins".

According to Reddy et al.,[34] c-phycocyanin from *Spirulina platensis* is a selective inhibitor of COX-2 with a very low IC_{50} COX-2/IC_{50} COX-1 ratio (0.04). These researchers used two related assays (isolated enzyme assay and human whole blood assay) to demonstrate selective COX-2 inhibition by c-phycocyanin. In the isolated enzyme assay, they found an IC_{50} that was much lower (180 nM) as compared to those of celecoxib (255 nM) and rofecoxib (401 nM). The latter are well-known selective COX-2 inhibitors that have become one of the biggest-selling pharmaceuticals in modern history. In the human whole blood assay, they demonstrated that phycocyanin effectively inhibited COX-2 with an IC_{50} value of 80 nM even lower than the value obtained for the partially purified enzyme. The apoprotein component of phycocyanin was responsible

for the inhibition of COX-2 since reduced phycocyanin and phycocyanobilin were found to be ineffective. The authors suggest that the hepatoprotective, anti-inflammatory, and anti-arthritic properties of phycocyanin reported in the literature might be due, in part, to its selective COX-2 inhibitory property, though they did not exclude a similar effect of phycocyanin through its ability to efficiently scavenge free radicals and inhibit lipid peroxidation.

Jain et al.[35] studied the effect of *Spirulina* purified protein (SPP) containing c-phycocyanin and other well-known COX-2 inhibitors (NS-398, rofecoxib) in LPS-mediated hyperlagesia. Acute administration of LPS (50 μg/mouse, i.p. or 10 μg/paw, i.p.) significantly demonstrated hyperalgesia in chemical, thermal and mechanical nociception. Treatment with SPP 50 and 100 mg/kg, p.o.), NS-398 (10 mg/kg, p.o.) and rofecoxib (10 mg/kg, p.o.) exhibited significant anti-hyperalgesic effect in chemical hyperalgesia. This study confirms the participation of inducible COX-2 enzyme in nociceptive conditions and the role of *Spirulina* phycocyanin as a COX-2 inhibitor.

Very recently, Reddy et al.[36] demonstrated that c-phycocyanin inhibited apoptosis of LPS-stimulated RAW 264.7 macrophages. Treatment of RAW 264.7 cells with phycocyanin (20 μM) significantly decreased cell viability after 48 hours. Phycocyanin also decreased the levels of PGE2 in a dose-dependent manner reaching maximum inhibition (90%) at 100 μM concentration. The decrease in PGE-2 was believed to be due to inhibition of COX-2. Confocal microscopic studies as well as flow cytometric studies confirmed the induction of apoptosis by phycocyanin in LPS-stimulated RAW 264.7 macrophages. Inhibition of enterovirus 71-induced apoptosis by allophycocyanin from *Spirulina* has also been reported recently.[37] These findings are significant in the context of the therapeutic application of phycocyanin in the treatment of cancer. Indeed selective COX-2 inhibitors are currently the subjects of intensive research in the area of cancer.

CONCLUSION

The anti-inflammatory effects of phycocyanin shown above in studies involving several models of inflammation and cellular function point to its great potential in the natural and therapeutic prevention and intervention in some degenerative and chronic diseases like cancer and heart diseases and age-related degenerative diseases like Alzheimer's disease, Parkinson's disease, arthritis, diabetes, macular degeneration and cataracts. It should be pointed out here that the antioxidant properties of phycocyanin shown in these studies have also been confirmed in studies involving oral administration of whole *Spirulina* at doses about 4 times higher than phycocyanin.[33] This is not surprising since the content of crude phycocyanin in *Spirulina* is about 20%. The search for natural NSAIDs is going on intensively. Several natural COX-2 inhibitors are already in the market. The evidence for the anti-inflammatory and COX-2 inhibitory effects of *Spirulina* and/or its extracts is so strong that such a potential application is not far-fetched.

Antioxidant activity is not a single step process. Rather it is a redox cascade involving many intermediate electron transport reactions that eventually result in the quenching of the radical or rendering it safer. Traditional natural approaches to disease intervention rely on synergy between various phytochemicals found in plants. In this respect *Spirulina* offers many phytochemicals that can work in synergy to elicit the antioxidant effects. In addition to phycocyanin, *Spirulina* contains mixed carotenoids including, among others, β-carotene and zeaxanthin that are known to scavenge reactive oxygen species, and superoxide dismutase which catalyses the dismutation reaction of the toxic superoxide radical. *Spirulina* also contains polysaccharides that are important in immune regulation and cancer.

Conventional wisdom has it that we eat colorful foods that are rich in antioxidants and phytochemicals. This is the basis for the 5-A-Day Program. In this respect *Spirulina* provides one part of the color spectrum that is not found in any common food, the blue color of phycocyanin. Whole *Spirulina* should be as effective as phycocyanin since the latter is soluble in water and is therefore expected to be readily available in the GI. The fate of *Spirulina* or phycocyanin in the GI is unknown. However it is clear from several studies that some components of *Spirulina* elicit their effects quite remote from the site of uptake in some cases passing the blood-brain barrier.[26] However, should phycocyanin be the product for choice for such applications, the other components remaining after its extraction such as the carotenoids, polysaccharides and some of the remaining amino acids can also be put to nutritional and therapeutic use.[4]

Increased cost of health care has become a driving force in the shift towards interest in wellness, self-care, and alternative medicine, and a greater recognition between diet and health care. The safety of *Spirulina* is well-established through several toxicological studies and through centuries of human use and it is already used widely as food and dietary supplement. The technology for mass production of *Spirulina* is well-developed and there are already several companies that produce it commercially.[1] Further research into the health benefits of *Spirulina* in general, and its antioxidant and anti-inflammatory effects in particular, should therefore be rewarding.

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