Nutrition and Dietary Supplements

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REVIEW

The beneficial effects of spirulina focusing on its immunomodulatory and antioxidant properties

This article was published in the following Dove Press journal:
Nutrition and Dietary Supplements
29 July 2010
Number of times this article has been viewed

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Abstract: Spirulina, linking bacteria and plants is primitive, has a simple structure but a complex composition. It has been a common dietary substance around the world from ancient times. Although dietary usage and supplementation continues to be popular, there was for a long time no strong scientific evidence of spirulina's nutritive and health benefits. In recent years, spirulina has attracted scientific attention, not only for its various health benefits, but also at a micro level of understanding the mechanisms of action of its various components. From being a 'complete' protein source, spirulina and its components have been shown to have positive benefit across a range of human health indications from malnutrition to antioxidant properties. These reports come from *in vitro*, animal and human studies. Although, few adverse effects of spirulina supplementation have been reported, most of these can be addressed by 'organic' production, good culture, harvest and processing practices along with its careful usage in specific conditions such as metabolic disorders. Case reports on effects of spirulina supplementations are many and with a larger evidence base of scientific validation studies, spirulina has the potential to be accepted by global accreditation/certification/approval authorities as a safe nutritional and dietary supplement.

Keywords: dietary supplement, immunomodulation, malnutrition, lipid modulation

Introduction

Human nutritional and dietary requirement understanding and an optimal provision of the same are of primary importance. Changing lifestyles, dynamic restructuring of micro and macro niches, and unavailability of nutrition sources contribute to an increasing incidence of malnourishment and other health risks. As the source for most nutritional requirements is the diet, it is necessary to look into the aspect of supplements that will boost the health status of individuals. Given this current scenario, it is necessary to find a way to provide cost-effective nutritional and dietary supplements. One supplement source, with ease of production, processing, distribution along with a wide range of macro- and micronutrients of human health benefits is spirulina. The number of research articles discussing the beneficial effects of spirulina is increasing every year. Also, the number of indications where spirulina is being utilized as an ideal dietary supplement is growing. In fact, the first two months of this year, saw about seven publications on spirulina in indexed journals. The exact origin of spirulina is still unexplained, but it is known to have appeared 3.6 million years ago as an evolutionary bridge between bacteria and green plants. Spirulina continues to be renewing itself over years, and now occurs almost ubiquitously across the globe and has been a rich

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source of nourishment to many cultures including America, Africa and the Middle East from ancient times.

It has been stated by NASA that the nutritional value of 1000 kg of fruits and vegetables equals one kg of spirulina. Therefore in long-term space missions NASA (CELSS) and European space agency (MELISSA) proposed that spirulina serves as a major source of food and nutrition.[1,2] The United Nations has hailed spirulina as the possible "best food for the future" in its world conference held during 1974. Spirulina can be harvested by simple methods and can be processed into a variety of final forms such as powders, tablets, flakes, syrups, etc.[3]

Some of the early health effects of spirulina were in its role in diabetes management and its significant plasma triglycerides reduction effects (total- and LDL-cholesterol), blood pressure lowering, improving the antioxidant status, as well as inflammatory effects.[4] Recent reports note the importance of spirulina for its immunomodulatory, anti fatigue and radio protective effects. Spirulina is commonly used in Asian cuisine. In America, spirulina is sold in health food stores as a powder or tablet. In Russia, it has been approved to treat symptoms of radiation sickness, because the carotenoids it contains absorb radiation.[5] Spirulina also is reported to slow neurological damage in aging animals, and also to lessen the damage caused by stroke.[6] Studies also show that spirulina can prevent the release of histamines, treating allergy symptoms.[7] The melanosis and keratosis improving capacity of Spirulina has also been demonstrated.[8]

The first documented report on spirulina dates back to the 16th century and spirulina is believed to have been a nutritional source for the Aztecs and Mesoamericans.[9] Large scale commercial production started in the early 1970s in an establishment run by Sosa Texcoco.[3] Spirulina occurs naturally in tropical and subtropical lakes with high pH and high concentrations of carbonate and bicarbonate. The largest concentrations of spirulina today can be found at Lake Texcoco in Mexico, around Lake Chad in Central Africa and along the Great Rift Valley in East Africa. Spirulina has been exhaustively and extensively tested by scientists around the world, and is reported to be a powerful and well-balanced source of nutrition. Arthrospira platensis occurs in Africa, Asia and South America, whereas Arthrospira maxima is confined to Central America.[3]

Spirulina is a blue-green algae due to the presence of both chlorophyll (green) and phycocyanin (blue) pigments in its cellular structure. The fresh-water ponds and lakes it favors are notably more alkaline (in the range of pH 8 to 11) than ordinary lakes and cannot sustain any other forms of microorganisms. In addition, Spirulina thrives in very warm waters of 32°C to 45°C (approximately 85°F to 112°F), and has even survived in temperatures of 60°C (140°F) It has photosynthetic activity and therefore is an autotroph. Being gram negative it has a complex cell wall composed of peptidoglycan. The helical shape of the trichome is characteristic of the genus.[5]

Spirulina production involves three major steps, viz., cultivation, harvesting and processing. Selected strains are used for cultivation of alga in specially constructed ponds. Most cultivated spirulina is produced in open-channel raceway ponds, with paddle-wheels used to agitate the water. The United States, Thailand, India, Taiwan, China, Pakistan and Burma are the largest commercial producers of spirulina.[5] Spirulina has many essential and nonessential macro- and micronutrients that makes it an ideal nutritional and dietary supplement (Table 1).

We review here, the beneficial effects of spirulina as a nutritional and dietary supplement covering all major areas of health benefits, with special focus on its immunomodulatory and antioxidant effects. We have considered for our review the information obtained from scientific publications with evidence based methods and data analysis. The databases utilized for obtaining information are scientific research publications from journals indexed/available through PubMed, Scopus, and GoogleScholar. Relevant details were also obtained from general databases such as Google. The key words used for the information search include 'Spirulina', 'S. platensis', 'dietary supplements', 'natural sources', 'immunomodulation', 'antioxidant' and 'benefits'. Both reviews and papers discussing a specific supplementary effect of spirulina were utilized for this review.

Nutritional supplementation

One health problem that is of great concern, especially in developing countries, is malnutrition. Severe forms of malnutrition are expressed as protein energy malnutrition defects such as kwashiorkor, marasmus and marasmic kwashiorkor. Apart from protein deficiencies, affected children usually do not have a complete balanced diet which includes the micronutrients such as vitamins and minerals that are essential for normal growth and development. The consequences of malnutrition represent a global problem, which affects morbidity as well as mortality. Increased tissue production of prostaglandin E_2 as a result of high intake of linoleic acid in a polyunsaturated fatty acid deficient diet, causes inhibition of the proliferation and cytokine production of Th1

Table 1 The major components of spirulina with nutritional importance making it an ideal dietary supplement

Nutrient	% composition	Details
Protein	65	All 8 essential amino acids: isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophane and valine 10 nonessential amino acids: alanine, arginine, aspartic acid, cystine, glutamic acid, glycine, histidine, proline, serine and tyrosine.
Carbohydrates	15	
Lipids	6	Gamma-linolenic acid (GLA), alpha-linolenic acid (ALA), linoleic acid (LA), stearidonic acid (SDA), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and arachidonic acid (AA).
Vitamins	0.75	thiamine (B1), riboflavin (B2), niacin (B3), pyridoxine (B6), folic acid (B9), cyanocobalamin (B12), biotin (B7), vitamin D, pantothenic acid (B5), vitamin E (tocopherol), inositol.
Minerals	8	Potassium, calcium, chromium, copper, iron, magnesium, manganese, phosphorus, selenium, sodium, and zinc.
Carotenoids	346 mg/100 g (variation noticed according to the processing methods)	Alpha-carotene, beta-carotene, xanthophylis, cryptoxanthin, echinenone, zeaxanthin and lutein.
Other pigments		Chlorophyll, phycocyanin, porphyrin, phycoerythrin, tetrapyrrole and phytonadione.
Moisture	3.80	

Notes: Spirulina also contains other biomolecules such as rhamnose sugars, trace elements and enzymes.

cells, the mediators of cellular immunity.[10] Diet-associated inhibition of the Th1 subset is a major contributor to the high prevalence of these diseases in sub-Saharan areas.[11] Spirulina is rich in proteins, carbohydrates, polyunsaturated fatty acids, sterols and some more vital elements such as calcium, iron, zinc, magnesium, manganese and selenium. It is a natural source of vitamin B12, vitamin E, ascorbic acid, tocopherols and a whole spectrum of natural mixed carotene and xanthophylls phytopigments. Spirulina as a supplement serves to provide these nutrition requirements and seems to be a 'wonder food'.

Role in diabetes mellitus

Diabetes mellitus (DM) is one of the most prevalent diseases and is of great concern globally owing to its health and socioeconomic repercussions. Diet plays a central key role in maintaining the blood glucose levels in diabetic patients to prevent complications arising. As spirulina has been associated with cholesterol regulatory, antioxidant and immune modulatory properties, it seems to be helpful to diabetic patients as a functional food. Spirulina helps in maintaining the nutritional balance in such chronic conditions.[4] Considering the critical lipid profile in DM patients, spirulina has been reported to have blood lipid lowering effects which have a positive impact on both healthy subjects as well as heart patients. Since dyslipidemia, oxidative and inflammatory stress are considered to be the contributing factors for diabetes, spirulina has great promise as a functional food for management of type 2 diabetes.[4] A study with diabetic rat models concluded that *Spirulina maxima* was effective in correcting the abnormal carbohydrate and lipid metabolisms caused by excess fructose within the body.[12]

Anticancer properties

The understanding of mechanisms of carcinogenesis and various other aspects of tumor biology is quite advanced and is of great importance with immediate and future implications. Two separate but extensively interrelated pathways leading to cellular apoptosis have been characterized as the extrinsic and the intrinsic pathways.[13] The extrinsic pathway is initiated by ligation of transmembrane receptors to activate membrane proximal "activator" caspases, which in turn cleave and activate downstream "effector" caspases. The intrinsic pathway requires disruption of the mitochondrial membrane and the release of mitochondrial proteins, two events that are regulated by the opposing actions of pro- and antiapoptotic Bcl-2 family members. The multifunctional transcription factor p53 is thought to be part of a "fast track" connection between nuclear DNA damage and the intrinsic pathway machinery.[14] p53 regulates multiple responses to genotoxic stress by transcriptional activation or repression of a number of genes encoding proteins involved in cell cycle control (p21WAF1/Cip1), DNA repair and apoptosis.[15] p21 (WAF1) is a CKI that directly inhibits the activity of cyclin D/CDC 2 and cyclin D/CDK 4 complexes. p21 functions as a regulator of cell cycle progression at S phase. The expression of p21 is controlled by the tumor suppressor protein p53. Sometimes,

Table 2 Immunomodulatory potentials of Spirulina in innate and adaptive immune responses along with its role in hypersensitive reactions

Immune response type	Mechanism of action/effects	References
Innate immunity	Expression of genes encoding chemokines IL-8, IL-1 β, IL -2, TNF-α, NF-kappa B, MCP-1, MIP-1α, MIP-1β, IP-10, enzyme cyclo-oxygenase-2 and THP-1 activation.	25, 36, 34
	Increase in the antigen-specific, as well as the total, IgA antibody level in the Peyer's patches, mesenteric lymph nodes and intestinal mucosa as well as in the spleen cells in mice.	37
	Lower antigen-specific IgG1 and IgE antibody levels in the serum suppressing allergic reactions.	
	Signaling responses through Toll in blood cells; increasing activity of macrophages, Natural Killer cells (NK), and neutrophils. The presence of co-operative IL-12 and IL-18 for NK-mediated IFN production.	34, 41
	Caspase dependent apoptosis induction in HeLa cells in vitro; activation of pro-apoptotic gene and down regulation of anti-apoptotic gene expression, to facilitate the transduction of tumoural apoptosis signals; activation of caspases 2, 3, 4, 6, 8, 9, and 10.	43
Innate immunity/hypersensitive reactions	Modulating the differentiation of Th2 cells mediated, in part, by inhibiting the production of IL-4 in patients with allergic rhinitis.	7, 39, 54
	Suppression of antigen specific IgE antibody. Reduces LTB4 and prostaglandin E2 levels in the arachidonic acid-induced mouse ear inflammation test.	
Hypersensitive reactions	Dose-dependent inhibition of histamine release from activated rat peritoneal mast cells.	55
	Increasing the levels of cyclic AMP inhibiting mast cell-mediated immediate-type allergic reactions *in vivo* and *in vitro*.	
Adaptive immunity	Th-1 type response and potentiating cell-mediated immunity; Antigen presenting cells.	33
	Modulating the T-cell subtype levels in adult and aged mice.	30
	Changes in leukocyte subset proliferation and cytokine productions responsiveness to two recall antigens, Candida albicans (CA) and tetanus toxoid (TT), *in vitro*.	32
	Potent antiviral properties against herpes simplex virus type 1, cytomegalovirus, influenza virus and human immunodeficiency virus type 1. Ca-SP has very low anticoagulant activity and long half-life.	28
	Increased humoral primary immune response to sheep red blood cells.	31, 43, 56
	Increases the percentage of phagocytic cells in peritoneal macrophages.	
	Enhanced mitogenic proliferation of lymphocytes	

it is expressed without being induced by P53. This kind of induction plays a big role in p53 independent differentiation which is promoted by p21.[16] Active ingredients in spirulina, either alone or in combination with certain other compounds are studied for antitumor activities and their role and mechanisms of actions well described; through various pathways outlined above.

Selenium-enriched Spirulina platensis extract (Se-SE) inhibited the growth of MCF-7 human breast cancer cells through induction of G1 cell cycle arrest and mitochondria-mediated apoptosis. This was also associated with a decrease in expressions of cyclin D1, cyclin D3, CDK4 and CKD6, and an increase in protein levels of p15 INK4B, p21 Waf1/Cip1 and p53. The synergistic effects include DNA fragmentation and nuclear condensation accompanied by the activation of caspase-8 and caspase-9 including PARP cleavage. The anticancer effects were induced by mitochondrial dysfunction through upregulation of Bax (Bcl2-associated X-protein) and Bad expression and downregulation of Bcl-xl expression.[17] C-phycocyanin (C-PC) showed downregulation of the antiapoptotic protein Bcl-2 and upregulation of the proapoptotic Bax protein in the R-HepG2 cells.[18] Calcium spirulan (Ca-SP) is a sulfated polysaccharide chelating calcium and is mainly composed of rhamnose. Ca-SP could reduce the lung metastasis of B16-BL6 melanoma cells, by inhibiting the tumor invasion of basement membrane. This activity was attributed to the preventive effect of adhesion and migration of tumor cells to laminin substrate and of the heparanase activity.[19] C-PC showed anticancer effects on human chronic myeloid leukemia cell line (K562). The effects include a significant decrease (49%) in the proliferation of K562 cells treated with 50 microM C-PC up to 48 h. Studies also revealed characteristic molecular and morphological features and fragmentation pattern typical for apoptotic cells. Downregulation of antiapoptotic Bcl-2 with no alterations in propoptotic Bax thereby tilting the Bcl-2/Bax ratio towards apoptosis was observed.[20]

Oral administration of spirulina at a dose of 800 mg/kg b.w. was shown to induce an adjuvant effect along with BCG-cell wall skeleton to augment antitumor natural killer (NK) cell activation in mice.[21] Also, in similar studies, a significant reduction in the hepatic cytochrome P-450 content and

Table 3 Antioxidant properties of Spirulina and their mechanisms

Component	Mechanism of action/effects	References
Phycocyanobilin – a derivative and homolog of biliverdin	Appearing to mimic the potent inhibitory impact of biliverdin and free bilirubin on NADPH oxidase activity	45
	"full-spectrum antioxidant therapy" (FSAT) that features a complementary array of natural antioxidants.	44
	Significant increase in exercise performance, fat oxidation, and GSH concentration and attenuated the exercise-induced increase in lipid peroxidation.	49
Protein extract containing phycobiliproteins	Showed a protector anti teratogenic effect in a dose-dependent manner.	50
	Protected in jaundiced rats against oxidative stress, as demonstrated by reduction of intestinal lipid peroxidation, increase of the antioxidant reduced glutathione (GSH), and decrease of the oxidized glutathione (GSSG).	51
Phycocyanobilin	Potent inhibitor of NADPH oxidase; broad range of anti-inflammatory, cytoprotective, and anti-atherosclerotic effects in rodents administered spirulina orally.	57
A fluorescent antioxidant holo-alpha-phycocyanin of Spirulina platensis with His-tag (rHHPC; recombinant holo-alpha-phycocyanin of Spirulina platensis with His-tag) in 5-l bench scale.	Scavenging hydroxyl and peroxyl radicals	58
Whole supplement	Significant increase in IL-2 level and superoxide dismutase activity in females.	25
Whole supplement	Significant decrease in lipid peroxidation (MDA) and elevation of levels of GSH, SOD, GPX, NO, creatinine and urea. Therapeutic potential in gentamicin sulphate induced nephrotoxicity.	59
Protein extract and the biliprotein phycocyanin	Protecting the activity of the cellular antioxidant enzymes GPx, GPx-Se and GR and by increasing reduced glutathione in cells against oxidative stress induced by iron. Mechanism related to antioxidant activity, capable of interfering with radical-mediated cell death.	47
Beta-carotene	Natural source compared to synthetic supplements; Vitamin A equivalence of spirulina beta-carotene in humans.	60
Ascorbic acid (AA) and water extract of *Spirulina plantesis* (SP)	Suppression of 5-fluorouracil induced lipid peroxidation to a significant extent.	61
Selenium and phycocyanin	Se-PC exhibited stronger antioxidant activity than phycocyanin by scavenging ABTS, DPPH, superoxide anion, and 2,2′-azobis-(2-amidinopropane)dihydrochloride free radicals. Dose-dependent protective effects on erythrocytes against H_2O_2-induced oxidative DNA damage. Potent antiproliferative agent against human melanoma A375 cells and human breast adenocarcinoma MCF-7 cells. Induction of apoptosis by DNA fragmentation, and nuclear condensation. Potent cancer chemopreventive activities.	62
Four selected Spirulina platensis preparations: (1) Biospirulina, (2) SpiruComplex, a preparation with naturally bound selenium, chromium and zinc, (3) SpiruZink, a preparation with naturally bound zinc, (4) Zinkspirulina + Acerola, a preparation with naturally bound zinc and acerola powder.	Dose-dependent inactivation of free superoxide radicals (antioxidant effect) as well as an anti-inflammatory effect – reduction of the metabolic activity of functional neutrophils and inactivation of superoxide radicals generated during an oxidative burst.	63
Whole supplement	Reducing the LPO level, serum glutamate oxaloacetate and serum glutamate pyruvate transaminase activity and increase in liver GSH level. Restoration of activities of antioxidant enzymes superoxide dismutase, catalase and glutathione-S-transferase to near normal level in mercuric chloride intoxicated mice. Defense mechanism in mercuric chloride induced toxicity and provides evidence that it may have a therapeutic role in free radical-mediated diseases.	64

(*Continued*)

Table 3 (*Continued*)

Component	Mechanism of action/effects	References
Chromophore phycocyanin, both without bound Se and as selenium-enriched.	Lowered plasma cholesterol and non-HDL cholesterol concentrations Significant increase in plasma antioxidant capacity. A sparing effect in liver glutathione peroxidase and superoxide dismutase activity. Cardiac production of superoxide anion significantly decreased The expression of p22phox subunit of NAD(P)H oxidase decreased Chronic consumption of Se-rich spirulina phycocyanin powerfully prevents the development of atherosclerosis. The underlying mechanism is related mainly to inhibiting pro-oxidant factors and at a lesser extent improving the serum lipid profile.	65
Whole supplement	Significant increase in exercise performance, fat oxidation, and GSH concentration and attenuated the exercise-induced increase in lipid peroxidation.	60
Spirulina and soy protein	Preventive effect of the skeletal muscle damage and that probably led to postponement of the time of exhaustion during all-out exercise.	67

enhancing hepatic glutathione S-transferase activity was observed in the group treated with spirulina in comparison with the control group.[22]

10 mg/day of *Spirulina platensis* extract, three times a week for 32 weeks was shown to slow down cancer progression in male golden Syrian hamsters exposed to 0.5% solution of 7,12-dimethylbenz[a]anthracene (DMBA) in buccal pouches.[23] Selenium-containing phycocyanin (Se-PC) showed potent antiproliferative properties in human melanoma A375 cells and human breast adenocarcinoma MCF-7 cells. Induction of apoptosis, accumulation of sub-G1 cell populations, DNA fragmentation, and nuclear condensation were noticed.[24] A positive effect on antioxidant enzymes viz., superoxide dismutase, catalase, glutathione reductase, glutathione peroxidase was demonstrated along with decreased skin and stomach tumor burden.[25]

In a first of its kind report, the potential use of Spirulina in chemoprevention of cancer has been demonstrated in dibutyl nitrosamine (DBN) induced rat liver toxicity and carcinogenesis. Spirulina supplementation prevented DMN induced severe liver injury and histopathological abnormalities. Also, spirulina supplementation reduced the incidence of liver tumors from 80% to 20%. Reduction of both PCNA and p53 were significant along with inhibition of cell proliferation, increased p21 and decreased Rb expression levels at 48 hrs post-treatment. In addition, SP increased Bax and decreased Bcl-2 expression, indicating induction of apoptosis by 48 hrs.[26]

Regression of hamster buccal pouch tumors has also been demonstrated following the local injection of alphatocopherol, canthaxanthin and an extract of Spirulina-Dunaliella algae. The study demonstrated that cancer regression was accompanied by a significant induction of tumor necrosis factor in macrophages in the tumor area, suggesting a possible mechanism of tumor destruction. Significant increase in TNF-α positive macrophages was found in animals with tumor-bearing pouches.[27] Animals fed canthaxanthin presented a notably and statistically significant reduction in tumor number and size compared with controls. Animals fed beta-carotene demonstrated a smaller but statistically significant reduction in tumor number and size. Animals supplemented with spirulina presented a complete absence of gross tumors. However, microscopic sections of the buccal pouch in the algae group showed localized areas of dysplasia and early carcinoma-*in-situ* undergoing destruction.[28] Regression of experimental hamster cancer was also demonstrated by supplementations with beta carotene and algae extracts.[29]

A cross-sectional study in Kuala Lumpur, Malaysia (where complementary alternative medicine [CAM] is in practice), was performed especially for pediatric cancers. 33% of CAM practitioners used spirulina to augment therapy (which plays an important role in sociocultural dimension of patients' health beliefs towards successful treatment).[30] In one of the first human studies, the chemopreventive activity of *Spirulina fusiformis* (SF) (1 g/day for 12 months) in reversing oral leukoplakia in pan tobacco chewers in Kerala, India has been reported. Complete regression of lesions was observed in 45% evaluable subjects supplemented with SF, as opposed to 7% in the placebo group. Within one year of discontinuing supplements, 9 of 20 (45%) complete responders with SF developed recurrent lesions. Supplementation with SF did not result in increased serum concentration of retinol or beta-carotene, nor was it associated with toxicity.[31]

The decreased expression of the phoshoprotein Rb, involved in regulating progression through the cell cycle and a concomitant increase in p21 expression indicate that these proteins along with p53 are important for spirulina driven inhibition of cell proliferation.[17] Spirulina is a protective phyto-antioxidant against liver toxicity and an antitumor agent. Although the *in vitro* and animal model studies indicate a potential application of spirulina supplementation, further preclinical and clinical trials are required to characterize the efficacy of spirulina in combination with existing therapeutics for chemoprevention and chemotherapy.[18] It was also reported to have prevented heart damage caused by doxorubicin without having an effect on its antitumor properties.[19]

Radioprotective properties

Concerns exist as to the unwarranted side effects of radiotherapy, chemotherapy and combined therapy, although they are currently the therapy modalities for many cancer types. Cancer treatments and management strategies are being constantly upgraded. Apart from novel therapeutics and drug delivery systems, several research contributions to this area stem from identification of suitable 'radio-protective' molecules/agents. This also has an impact in other areas such as occupational, accidental and intentional radiation exposures. It has been reported that spirulina promotes hematopoietic stem cells and progenitor cells to differentiate after lethal Co-γ radiation and thus increase the rate of survival in mice.[20]

Antiviral properties

An interesting study was carried out by preparing a hot water extract of spirulina and subjecting it to fractionation. A part of the fractionated product was found to inhibit the replication of several viruses, especially those with an envelope such as the measles virus, and the HIV-1 virus, in human T cells, peripheral blood mononuclear cells and Langerhans cells. This component was found to be a sulfated polysaccharide, calcium spirulan.[21,22] In order to find out the chelating property of calcium in the inhibition of replication of virus, the calcium was replaced by sodium. As a result the antiviral property was inhibited. Therefore calcium was seen to play an essential role in a dose-dependent manner for inhibiting the cytopathic role of such viruses.[23] In addition, in undernourished children spirulina has been found to improve weight gain and correct anemia in both HIV-infected and HIV-negative cases.[24]

Lipid-modulating properties

In a randomized, double-blind, placebo-controlled intervention study, it was observed that spirulina showed a significant reduction of the low-density lipoprotein (LDL) to high-density lipoprotein (HDL) ratio after four months of supplementation.[25] In rodents, the water soluble fraction of spirulina significantly reduced the LDL/HDL ratio.[26] Spirulina supplementation decreased the levels of plasma lipid concentration and modified the total cholesterol and HDL cholesterol levels. Spirulina also was reported to decrease blood pressure by promoting vasodilation and restricting vasoconstriction.[27]

Immunomodulatory properties

The aqueous extract of spirulina was found to have a major impact on the immune system by increasing the phagocytic activity of macrophages, stimulating the NK cells. It also played a role in the activation and mobilization of T and B cells due to its stimulatory effects in the production of cytokines and antibodies.[28] It also was beneficial to the transplant community as this extract causes suppression of CD28 and co-stimulation of human T cells to the extent of the same immunosuppressive effect produced by drugs like cyclosporine which have a lot of side effects.[29] The photosynthetic pigment phycocyanin has a part to play in modulating the immune system. It has been seen to have an inhibitory effect on the release of histamine from mast cells during an allergic inflammatory response.[20] It has also been noted that in *in-vivo* and *in-vitro* it suppressed the growth of tumor cells, promoted NK cell activity and induced the lymphocytes in spleen to produce TNF-α.[20]

Using flow cytometry, analysis age-related changes were found in the intestinal intraepithelial lymphocytes along with their functional preservation, by feeding Spirulina to mice. Cells having the leucocyte common antigen $CD45^+$ were used as representative markers for IELs. There was a significant increase in the proportion of $CD45^+CD8^+$ cells in aged mice, while it was lower in adult mice. Also the $CD4^+CD8^+$ cells of adult mice were greater than that of aged mice. When aged mice were fed with spirulina there were changes in the level of these cell surface antigens. This indicates that spirulina contributes to the functional preservation of the intestinal epithelium which acts as a first line of mucosal barrier against infections.[30]

In a study done with another strain of spirulina (spirulina fusiformis) in vivo effects on inhibition of humoral immune response, cell mediated immune response (delayed type hypersensitivity) and TNF-α were noticed in a dose-dependent manner in mice. The *in vitro* effects were seen as a decrease in the mitogen-induced T lymphocyte proliferation. This suggests the immunosuppressive effects of spirulina and can

provide a strong scientific validation as its use as a drug.[31] Spirulina is also seen to elicit strong IL-1β, IL-responses which cause an age dependent, temporary enhancement of the adaptive immune response. It was noted that spirulina was more effective in potentiating the cell-mediated immunity by stimulating the Th-1 type response. The morphologic changes and morphometric investigations of the spleen in rats confirmed the immunostimulatory effect of spirulina by NBT test of peritoneal macrophages.[32,33] Spirulina can enhance components of the mucosal and systemic immune system as it activates the cells of innate immune system. When a cytokine array test was performed, it was noted that spirulina had an increased effect on the expression of genes encoding the chemokines interleukin (IL)-8, MCP-1, MIP-1α, MIP-1β, IP-10, the cytokines TNF-α, IL-1β, and the enzyme cyclo-oxygenase-2 (COX-2). THP-1 activation was confirmed by measuring immune cytokine mRNA induction using reverse transcriptase-polymerase chain reaction (RT-PCR).[34–36]

The effects of phycocyanin (a biliprotein of Spirulina) is seen to enhance the biological defense activity by reducing allergic inflammation by the suppression of antigen specific IgE antibody and through maintaining the mucosal immune system function against infectious diseases in C3H/HeN and BALB/cA mice.[37] Spirulina has also been found to protect against hay fever.[38] In allergic rhinitis patients, a more recent double-blind, placebo-controlled study showed marked reduction in the secretion of proinflammatory IL-4 by 32% along with symptomatic relief on supplementing them with spirulina platensis.[40] It was also seen to reduce inflammation in arthritis patients and this reduction was due to the stimulation to secrete IL-2 which regulates inflammatory response.[25]

The immunostimulatory effects of spirulina on TNF-α play a major role in precipitating autoimmune diseases in genetically predisposed persons. When blood cells were stimulated with BCG cell wall skeleton in volunteers, those exposed to spirulina had a more potent IL-12 p40 production than those who were not. This was explained due to the direct effect on myeloid lineages. BCG cell wall skeleton serves as a ligand for toll-like receptors (TLR) 2 & 4 to upregulate the maturation stage of monocytes/macrophages, Spirulina administered orally is effective as it is involved in the signaling response through Toll in blood cells. Spirulina significantly acts indirectly on NK cells as it is critically important for NK-mediated IFNγ production.[40,41] Several immunological functions such as NK cell activity were seen to show a two fold increase by spirulina supplementation at 10,000 ppm as dietary inclusion in Cornell K-strain White Leghorns and broiler chicks.[42] The primary response to sheep red blood cells was increased due to the production of splenic antibody producing cells in spirulina fed mice though the IgG Ab production in secondary response was rarely seen (Table 2).[43]

Antioxidant properties

During oxidative stress, changes occur in NADPH activity, resulting in differences in the conformation of enzymes involved in the mitochondrial respiratory chain, nitric oxide synthase, xanthine dehydrogenase thereby generating high levels of superoxide. This leads to apoptosis, cardiac remodeling due to chronic pressure overload, atrial fibrillation etc. The effect of bilirubin and biliverdin on NADPH oxidase activity is mimicked by a phytochemical phycocyanobilin which is a homolog of bileverdin.[44] The key mediators of vasculopathies in sickle cell anaemia are due to loss of nitric oxide and oxidative stress. A phytochemical richly present in spirulina, phycocyanobilin reportedly inhibits NADPH oxidase activity and promotes glutathione synthesis along with a marked production of antioxidant enzymes having potential for management of oxidative stress in sickle cell disease.[45] Analytical methods for profiling compounds in spirulina responsible for antioxidation, allowed a complete characterization of chemical and biochemical mechanisms of action.[46] The iron chelating properties of spirulina was discovered when human neuroblastoma cells *in vitro* were exposed to toxic amounts of iron and then to spirulina, which revealed that the iron induced oxidative stress was reduced.[47] Geriatric patients administered spirulina for 16 weeks showed a remarkable improvement in the antioxidant potential, as measured by the increased levels of antioxidant status in plasma of these individuals.[25] A double-blind, placebo-controlled study performed on individuals after exercise, showed decreased amount of creatine kinase, (an indicator of muscular breakdown) when they were supplemented with spirulina. Moreover their exhaustion time in the treadmill exercise increased by 52 seconds. This could be explained by the antioxidant potential of spirulina.[48] There was an attenuation in the exercise induced increase in lipid peroxidation, as spirulina supplementation had a marked increase in exercise performance, fat oxidation and GSH concentrations.[49]

Teratogenesis is a threat, as it results in congenital malformations which are the major cause of child mortality in the world. Reactive oxygen species is one of the major teratogenic mechanisms leading to these malformations. A study of the antioxidant effect of spirulina was carried out by treating pregnant mice with hydroxyurea, a teratogenic antineoplastic

drug. It was reported that spirulina showed its protection in a dose-dependent manner as an antioxidant.[50] In obstructive jaundice, alteration in the altered intestinal barrier function is one important mechanism resulting in endotoxemia and it was found that spirulina had significant protective effects on mucosal barrier by reducing the intestinal translocation of bacteria and endotoxin.[51] Spirulina reduces the severity and recovery of strokes. It also reverses age-related declines in memory and learning (Table 3).[52,53]

Conclusion

Spirulina has potential for being a 'wonder food supplement' and several leading organizations have praised its beneficial effects. In spite of the wide usage for the beneficial effects, a few side effects and contraindications have been reported with spirulina use. The most frequently reported adverse effects are headache, muscle pain, flushing of the face, sweating, and difficulty concentrating. These have been described in people taking 1 g of spirulina orally. Skin reactions have also been reported. Spirulina should be avoided by people who have the metabolic disorder phenylketonuria, as phenylalanine content can be harmful.[55] Although rare, cyanobacteria like Spirulina may contain toxins called microcystins, which accumulate in the liver and can potentially cause cancer or other liver diseases. One concern is the culture conditions of the spirulina that is being utilized. The presence of harmful elements, toxic substances, etc will accumulate in the biomass and can pose serious human health effects. Blue-green algae harvested in uncontrolled culture conditions may be contaminated with heavy metals that can lead to liver damage, diarrhea, and vomiting. One approach to circumvent this is to grow spirulina in an 'organic' way. USFDA's National Organic Standard Boards have guidelines to produce spirulina as 'organic' and also certifies the same. Such organic certifications will alleviate the concerns about spirulina being a safe vegetarian nutritional and dietary supplement. A wealth of information available based on scientific studies and more evidences from validation studies should see spirulina into the various phases of clinical trials to meet the requirements of USFDA and also for its approval as a certified, approved nutritional and dietary supplement.

Acknowledgment

We acknowledge Mrs MD Sujatha for her editorial assistance and formatting inputs towards this manuscript preparation.

Disclosure

The authors report no conflicts of interest in this work.

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