CONTENT

Focus on

Essential amino acids

An amino acid is a chemical unit that enables the cells to maintain their structure by providing them with all the necessary building material. The term amino acid comes from the fact that it contains an amino group (NH2) and an acidic carboxyl group (COOH). Essential amino acids can't be produced by the body and must be derived from food.

There are 9 essential amino acids:

Histidine: This amino acid is a precursor to histamine, which is responsible for a wide range of physiological processes. Histidine increases calcium absorption, reduces histamine levels, and in turn controls diarrhea. Isoleucine : isoleucine is important for the regulation of blood sugar. Leucine : Leucine is one of three essential amino acids that increase muscle mass and helps muscle recover after exercise. It also regulates blood sugar and supplies the body with energy. Leucine is used clinically to help the body heal, and it also affects brain function. Lysine : lysine enables the synthesis of carnitine, which converts fatty acids into energy and also plays an important role in the production of hormones, antibodies and enzymes. Methionine: this amino acid aids in the production of sulphur, which is necessary for normal metabolism and it is also essential for the synthesis of haemoglobin and glutathione that fights against free radicals. Phenylalanine: This amino acid is a precursor to catecholamines that regulate the central and peripheral nervous system. Threonine: this amino acid is needed to create other amino acids that aid the production of collagen. It is also important for antibody production. Tryptophan: tryptophan is the largest amino acid and is a precursor of serotonin and melatonin, which means that it can regulate mood and sleep. Valine: valine is necessary for muscle metabolism and the repair of tissues and can be useful in the treatment of liver and gallbladder disorders.

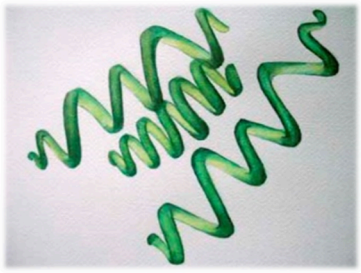
Calcium spirulan (Ca-SP)

This polysaccharide is composed of rhamnose, ribose, mannose, fructose, galactose, xylose, glucose, glucuronic acid, galacturonic acid, sulfate, and calcium.

Effect on viruses: Ca-SP was found to inhibit the replication of several enveloped viruses, including Herpes simplex virus type 1, human cytomegalovirus, measles virus, mumps virus, influenza A virus, and HIV-1. It was revealed that Ca-SP selectively inhibited the penetration of virus into host cells. Its potency at least comparable to that of acyclovir.

Effect on cancer: Ca-SP was found to inhibit the tumor Invasion and metastasis of both B16- BL6 melanoma cells, Colon 26 carcinoma and HT-1080 fibrosarcoma cells in a concentration-dependent manner.

1. What is Spirulina?



Spirulina is a microscopic blue-green alga that grows in alcaline water. It became famous after it was successfully used by NASA as a dietary supplement for astronauts on space missions.

Spriulina is rich in *amino acids, polysaccharides, essential fatty acids, minerals, vitamins, carotenoids, enzymatic pigments* and *enzymes*.

Some of its compounds, such as *phycocyanin*, *calcium spirulan (Ca-SP), superoxide dismutase (SOD)* and *sulphoquinovosyl diacylglycerol (SQDG)* make it particularly interesting for human health.

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2. Main health properties of Spirulina

Several positive effects on health have been documented, in clinical, observation, in vivo and in vitro studies:

- Immune properties
- Antiallergic properties
- Antioxidant properties
- Anticancer properties
- Anti-viral properties
- Antibacterial properties
- Digestive properties
- Anti-diabetes properties (blood sugar level and insulin resistance lowering properties)
- Cholesterol lowering properties
- Antihypertensive) (high blood pressure lowering) properties
- Hepatoprotective properties
- Protection against atherosclerosis
- Protection against heavy metals
- Protection against radiation

A brief selection of the available clinical studies is listed under point 4.

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3. Composition of Spirulina

3.1 Amino acids

Spirulina contains between *50 and 70% protein* in a *highly* digestible form, this is 2x higher than soya and 3x higher than meat or fish.

Spirulina is considered as a complete protein source, because it provides all of the 9 essential amino acids: Histidine, Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Threonine, Tryptophan and Valin. Essential amino acids cannot be synthesized by the body.

Spirulina also provides 9 out of 12 non essential amino acids. Alanine, Arginine, Aspartic acid, Cysteine, Glutamic acid, Glycine, Proline, Serine and Tyrosine.

3.2 Polysaccharides

Spirulina contains *10-15 % carbohydrates*, primarily polysaccharides, easily absorbed by human cells with minimal intervention of insulin. Hence, Spirulina sugars provide quickly energy, while minimizing the risk of hypoglycemia.

Spirulina contains specific sulfated polysaccharides, such as calcium spirulan (Ca-SP), with powerful antioxidant, antitumor and antiviral properties. Calcium spirulan, composed of rhamnose, ribose, mannose, fructose, galactose, xylose, glucose, glucuronic acid, galacturonic acid, sulfate, and calcium, was found to inhibit the replication of several enveloped viruses, including Herpes simplex virus type 1, human cytomegalovirus, measles virus, mumps virus, influenza A virus, and HIV-1.

Focus on

Gamma-linolenic acid (GLA)

Gamma linolenic acid is an omega-6 fatty acid, which the body can convert to substances that reduce inflammation and cell growth.

Gamma linolenic acid (GLA) is used for conditions that affect the skin including systemic sclerosis, psoriasis, and eczema. It is also used for rheumatoid arthritis (RA), polyps in the mouth, high cholesterol and other blood fats, heart disease, metabolic syndrome (Syndrome-X), diabetic nerve pain, attention deficit-hyperactivity disorder (ADHD), depression, depression after childbirth, chronic fatigue syndrome (CFS), and hay fever (allergic rhinitis). Some people use it to prevent cancer and to help breast cancer patients respond faster to treatment with the drug tamoxifen.

Sulphoquinovosyl diacylglycerol (SQDG)

Sulphoquinovosyl diacylglycerol is sulfated polysaccharide. Sulfated polysaccharides (SPS) extracted with hot water from Spirulina were found to be rich in sulfate contents with values 5.02 % and 4.13%), respectively.

The sulfated polysaccharides have exhibited strong antioxidant, antitumor, immunostimulatory, anti-inflammatory, pulmonary fibrosis anticoagulant /antithrombotic, lipid lowering, antiviral (they are potent and selective inhibitors of various enveloped viruses, including herpes simplex virus, cytomegalovirus, vesicular stomatitis virus, and human immunodeficiency virus), antibacterial, antiprotozoan, hyperplasia prevention, gastrointestinal, regenerative and nano medicine applications.

Provitamin A (beta-carotene) and vitamin B12

Spirulina is the richest source of beta-carotene. Human bodies convert beta carotene to Vitamin A only as needed. Vitamin A deficiency is one of the most serious malnutrition diseases in the developing world, leading to blindness. Beta carotene has therapeutic effects, including reducing serum. Cancer health authorities have published studies showing beta carotene may reduce risks of all kinds of cancers.

Spirulina is the richest source of vitamin B12, higher than beef liver, chlorella or sea vegetables. Vitamin B12 is necessary for development of red blood cells, especially in the bone marrow and nervous system.

3.3 Essential fatty-acids

Spirulina is made of only *7 % lipid*, most of it in form of essential fatty acids.

Spirulina is particularly rich in gamma-linolenic acid (GLA), it is the second richest source of this nutrient after maternal milk. GLA has showed positive effects on cardiovascular protection (lowering total cholesterol and LDL, raising HDL cholesterol), atopic dermatitis, immune system (increasing lymphocytes), rheumatoid arthritis and possibly cancer treatment.

Spirulina also contains sulfolipids, a class of lipids which possess a sulfur-containing functional group. Spirulina sulfolipids, such as sulphoquinovosyl diacylglycerol (SQDG) have proved to be effective against HIV. Preparations obtained from Spirulina biomass have also been found active against herpesvirus, cytomegalovirus and influenza virus.

3.4 Minerals

Spirulina contains essential minerals and trace elements absorbed from its growth medium into chelated, easily absorbed forms:

- Potassium : A crucial mineral that regulates body electrolyte balance. Deficiency can cause heart arrest, hypertension, adrenal exhaustion and muscular collapse.
- Calcium : The most abundant mineral in the body, it is especially important to bone and dental health, but is also involved in neural transmissions to the muscles. Spirulina supplies about as much calcium, gram for gram, as milk.
- Zinc : The pivot point of over thirty vital enzymatic reactions, with profound effects on mental health, skin tone, prostate function and healing capacity.
- Magnesium : Deficiency can lead to spasmodic muscle disorders, including cardiac irregularities. Helps assimilation of vitamin C, B vitamins and protein.
- Manganese : Activates enzyme systems, along with zinc. Promotes activity of neurotransmitter acetylcholine, and helps stabilize blood sugar.
- Selenium : Originally believed to be a toxic heavy metal, but now known to be necessary for health. It retards aging, harmful oxidation and free radical formation, reduces the toxic effect of carcinogens, and improves cardiac efficiency.
- Iron : Promotes formation of hemoglobin, the oxygen-carrying blood pigment found in healthy red blood cells. Iron deficiency is most common among women in their reproductive years.
- Phosphorus : The second most abundant mineral in the human body, it is found in practically every cell. Functions with calcium to maintain bone density. Helps to digest carbohydrates and the B vitamins niacin and riboflavin.

3.5 Vitamins

Spirulina supplies several of the vitamins that we need to carry on metabolic processes:

- Provitamin A (beta-carotene) : one gram of Spirulina covers 46 % the daily requirements in vitamin A for an adult (see also below under “carotenoids”). Provitamin A is antioxidant and essential to eyesight. It protects the skin and the eyes from UV and prevent skin aging. The level of beta-carotene in spirulina is 10x higher than in carrots.
- Vitamin B1 : for optimal functioning of our muscular and nervous system.
- Vitamin B2 : needed for energy production.
- Vitamin B3 : helps reducing cholesterol.
- Vitamin B 12 : Spirulina is the only non-animal source of vitamin B12. Its concentration is 4x higher than raw liver. One gram of Spirulina covers 33 % the daily requirements. Vitamin B12 fights anaemia. It is important for red blood cell creation and development. Spirulina is therefore highly recommended for vegetarian people.
- Vitamin E : has a positive effect on the cholesterol level and prevents cardiovascular diseases.
- Vitamin K : Increases calcium fixation, softens artery and increases blood coagulation.

Spirulina also provides the following vitamins : B5, B6, B7, B8, B9, and D.

3.6 Carotenoids

Carotenoids are organic pigments. They are used as precursors by our body to synthesize the appropriate vitamins.

A good example is provitamin A (beta-carotene), a precursor of vitamin A. The advantage of beta-carotene is that it is converted by our body into vitamin A only when needed, thus minimizing the dangers of toxicity linked with an overdose of vitamin A (difficult to eliminate).

Focus on

Phycocyanin

Spirulina has a dark blue-green color, because it is rich in a brilliant blue polypeptide called Phycocyanin, which makes about 14% of its entire weight.

Phycocyanin has several proven effects on health:

Stimulates blood production: Phycocyanin stimulates hematopoiesis, (the creation of blood) and regulates production of white blood cells.

Strengthens the immune system: Phycocyanin strengthens the body's resistance through the lymph system.

Scavenges free radicals: The chemical structure of phycocyanin is very similar to bilirubin. Phycocyanin is a powerful anti-oxidant & anti-hydroxyl radical (The most dangerous free radicals produced during chemotherapy, when exposed to hand phone radiation, deep fried foods). Phycocyanin also protect the cells against DNA damage.

Reduces lipid peroxidation: Phycocyanin significantly inhibits the lipid peroxidation.

Protects the liver: Phycocyanin protects and improves liver function.

Fights inflammation: Phycocyanin powerful anti-inflammatory properties.

Fights neuro-degenerative diseases: Phycocyanin has proven positive effects on neuro-degenerative diseases such as Alzheimer's and Parkinson's.

Ishii and al. showed a significant positive correlation between the total s-IgA level in human saliva and the total amount of Spirulina consumed.

Ishii K, Katoh T, Okuwaki Y, Hayashi O. Influence of dietary Spirulina platensis on IgA level in human saliva. J Kagawa Nutr Univ. 1999; 30: 27-33.

Spirulina and Antibody Production 219

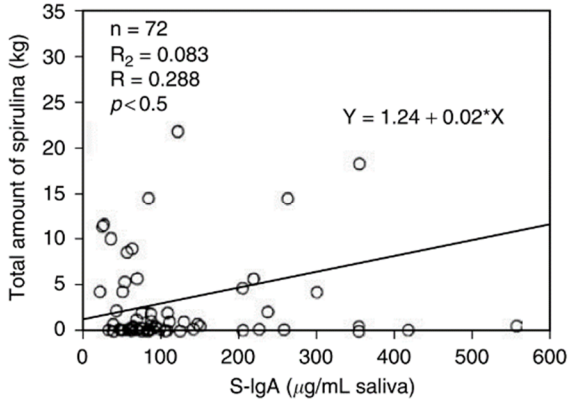

FIGURE 10.13 Correlation between total S-IgA levels in saliva of subjects and total amount of *Spirulina* ingested by subjects (From Ishii, K., *J. Kagawa Nutr. Univ.*, 30, 30, 1999. With permission.)

Spirulina contains carotenoids in the following forms: Alpha-carotene, Beta-carotene, Xanthophylis, Crypto-xanthin, Echinenone, Zeaxanthin, Lutein.

3.7 Enzymatic pigments

Spirulina is also rich in pigments that are bio-chemically important. Without theses pigments, our organism would not be able to synthesize many of the enzymes necessary for the balance of our metabolism.

- Chlorophyll: This pigment gives the green colour to spirulina. It helps human body to eliminate toxic heavy metals (mercury, arsenic, lead)
- Phycocyanin: This pigment gives its blue colour to spirulina. It stimulates the immune system, and has antioxidant as well as anti-inflammatory effects. *Spirulina is the only source of phycocyanin in the world*.
- Porphyrin: a red compound that forms the active nucleus of hemoglobin.

3.8 Enzymes

Besides enzymatic pigments, Spirulina contains many other enzymes. It is particularly rich in superoxide dismutase (SOD), a powerful antioxidant.

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4. Health properties of Spirulina

4.1 Immune properties

Our immune system is a complex system, involving specialized cells that communicate with each other via chemical messengers called cytokines. It protects us against pathogenic organisms like *bacteria, viruses, cancer cells, and parasites*, and against other compounds that are recognized as "foreign" or "non-self". Any cell or molecule recognized as non-self is attacked by our immune system cells and the antibodies they produce.

It has been established that nutrient deficiency can affect negatively our immune system: production of T-cells, lymphocyte response to mitogens and antigens, phagocyte function, secretory IgA anti-body response, NK cell activity and production of cytokines. It has also been established that some nutrients, such as the ones found in Spirulina, can enhance our immune function. The positive effect of Spirulina in this regard has been demonstrated in several studies.

Clinical studies

Hayashi et al. were the first to publish detailed studies on immunomodulatory properties of dietary Spirulina in mice. The authors concluded that Spirulina and its extract enhance the immune function through the modulation of macrophage function, phagocytosis and IL-1 production.

Hayashi O, Katoh T, Okuwaki Y. Enhancement of antibody production in mice by dietary Spirulina platensis. J Nutr Sci Vitaminol. 1994;40:431-441.

Ishii and al. showed a significant positive correlation between the total s-IgA level in human saliva and the total amount of Spirulina consumed.

Ishii K, Katoh T, Okuwaki Y, Hayashi O. Influence of dietary Spirulina platensis on IgA level in human saliva. J Kagawa Nutr Univ. 1999; 30: 27-33.

Saeki et al. showed that IFN- γ secretion activity and NK cell damage activities in human patients were significantly enhanced after two weeks of Spirulina extract administration. Surprisingly, the IFN- γ and NK cell activities continued up to 6 months after administration of extract was discontinued.

Saeki Y, Matsumoto M, Hayashi A, Azuma I, Toyoshima K, Seya T. The effect of Spirulina hot water extract to the basic immune activation. Summary of paper presented at the 30th Annual Meeting of the Japanese Society for Immunology. November 14-16, 2000.

Antiallergic (immunomodulative) properties of Spirulina

Li-chen Wu, Ja-an Annie Ho, Spirulina in human nutrition and health, Gerswhin M E and Belay A, CRC Press, 2008

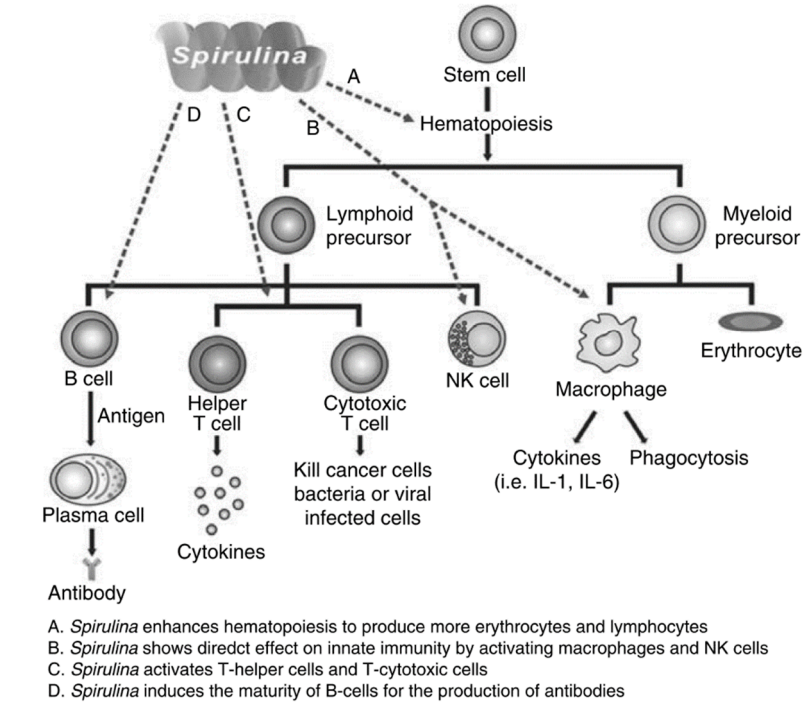

FIGURE 6.2 Effects of *Spirulina* on immunomodulation.

Focus on

Free radicals, oxydative process and antioxidants

Free radicals reactive oxygen species and reactive nitrogen species are generated by our body :

- *by various endogenous systems*: mostly by the respiratory system, but also by phagocytosis, prostaglandin synthesis etc.
- *by exposure to different physiochemical conditions*: cigarette smoke, environmental pollutants, radiations, certain drugs, pesticides, industrial solvents, ozone
- *by specific pathological states*: injuries, bacterial or viral infections etc.

A balance between free radicals and antioxidants is necessary for proper physiological function. If free radicals overwhelm the body's ability to regulate them, a condition known as oxidative stress ensues.

Free radicals thus adversely alter lipids, proteins, and DNA and trigger a number of human diseases. Hence application of external source of antioxidants can assist in coping this oxidative stress

Oxidative stress play an important role in many diseases and conditions: aging process, heart diseases, stroke, arthersclerosis, hypertension, arthritis, certain cancers, gastric ulcers, neurological disorder (Alzheimer's disease, Parkinson's disease) etc.

An antioxidant is a molecule able to neutralize free radicals, thus reducing its capacity to damage.

- Some antioxidants are produced by our body: glutathione, ubiquinol and uric acid.
- Other antioxidants come from our diet: B-carotene, Phycocyanin, GLA (found in Spirulina) and also vitamin E, vitamin C, selen, zinc etc.

4.2 Antiallergic (immunomodulative) properties

Although cytokine-induced responses are generally protective, an excess production and/or activity of cytokines can be harmful. Beside its positive immune properties, Spirulina also has the ability to modulate immune functions by inhibiting the release of histamine by mast cells and by enhancing IgA production. Spirulina has therefore a positive effect on allergies and on other autoimmune diseases.

Clinical studies

Yang et al., did extensive studies on the effect of orally-administered Spirulina on anaphylactic reaction on rats. They found that: 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-dinitrophenyl 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 factor α production from RPMC.

Yang H, Lee E, Kim H. Spirulina platensis inhibits anaphylactic reaction. Life Sciences. 1997; 61:1237-1244.

In a randomized, double-blind placebo-controlled trial, individuals with allergic rhinitis were fed daily, either with placebo or Spirulina for 12 weeks. The study showed that Spirulina significantly reduced IL-4 levels by 32%, demonstrating the protective effects of this microalga toward allergic rhinitis.

T. K. Mao, J. van de Water, and M. E. Gershwin, "E ff ects of a Spirulina-based dietary supplement on cytokine production from allergic rhinitis patients," Journal of Medicinal Food, vol. 8, no. 1, pp. 27–30, 2005.

This double blind placebo controlled study evaluated the effectiveness and tolerability of spirulina for treating patients with allergic rhinitis. The results showed that the consumption of Spirulina significantly improved both the symptoms and physical findings compared with placebo, including nasal discharge, sneezing, nasal congestion and itching. The conclusion was that spirulina is clinically effective on allergic rhinitis when compared with placebo.

Cingi C, Conk-Dalay M, Cakli H, Bal C. The effects of spirulina on allergic rhinitis. Eur Arch Otorhinolaryngol. 265(10):1219-23. doi: 10.1007/s00405-008-0642-8. 2008.

4.3 Antioxidant properties

The relationship between antioxidant intake and incidence of chronic diseases such as cancer, cardiovascular disease, cataracts, and premature aging that are associated with free radical damage is now well established through many epidemiological, intervention, and clinical studies.

Spirulina provides several carotenoid pigments with antioxidant properties, such as beta carotene (associated with cancer prevention), lutein and zeaxanthin (associated with prevention of age-related macular degeneration). Spirulina also contains around 7% phycocyanin (dry weight basis) and polysaccharides, both known to have powerful antioxidant properties. In addition, Spirulina in rich in superoxide dismutase (1,700 units/g), an enzyme that inhibits the oxygen radical generation.

The antioxidant properties of Spirulina have been demonstrated in numerous studies, in particular for phycocyanin, as Spirulina is the *only* of this rare blue pigment source in the entire world.

Clinical studies

Manoj et al. reported in an *in vitro* study that the alcohol extract of Spirulina inhibited lipid peroxidation more significantly (65% inhibition) than chemical antioxidants like α-tocopherol (35%), butylated hydroxy anisol (45%), and β-carotene (48%).

Manoj G, Venkataraman LV, Srinivas L. Antioxidant properties of Spirulina (Spirulina platensis). In: Seshadri and Bai. Spirulina. MCRC. 1992:48-154.

Romay et al. showed in what could be the first report on antioxidant and anti-inflammatory properties of phycocyanin, that phycocyanin was able *in vitro* to scavenge hydroxyl (IC 50 = 0.91 mg/ml) and alkoxyl (IC 50 = 76 µ g /ml) radicals with activity equal to 0.125 mg/ml of dimethyl sulfoxide (DMSO) and 0.038 µ g/ml of trolox, specific scavengers of those radicals respectively. Phycocyanin also inhibited liver microsomal lipid peroxidation (IC 50 =12 mg/ml).

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ORIGINAL ARTICLE

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Anti-cancer effects of blue-green alga *Spirulina platensis*, a natural source of bilirubin-like tetrapyrrolic compounds

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ABSTRACT

Spirulina platensis is a blue-green alga used as a dietary supplement because of its hypocholesterolemic properties. Among other bioactive substances, it is also rich in tetrapyrrolic compounds closely related to bilirubin molecule, a potent antioxidant and anti-proliferative agent. The aim of our study was to evaluate possible anticancer effects of *S. platensis* and *S. platensis*-derived tetrapyrroles using an experimental model of pancreatic cancer. The anti-proliferative effects of *S. platensis* and its tetrapyrrolic components [phycocyanobilin (PCB) and chlorophyllin, a surrogate molecule for chlorophyll A] were tested on several human pancreatic cancer cell lines and xenotransplanted nude mice. The effects of experimental therapeutics on mitochondrial reactive oxygen species (ROS) production and glutathione redox status were also evaluated. Compared to untreated cells, experimental therapeutics significantly decreased proliferation of human pancreatic cancer cell lines *in vitro* in a dose-dependent manner (from 0.16 g·L-1 [*S. platensis*], 60 μM [PCB], and 125 μM [chlorophyllin], $p<0.05$). The anti-proliferative effects of *S. platensis* were also shown *in vivo*, where inhibition of pancreatic cancer growth was evidenced since the third day of treatment ($p < 0.05$). All tested compounds decreased generation of mitochondrial ROS and glutathione redox status ($p = 0.0006$; 0.016; and 0.006 for *S. platensis*, PCB, and chlorophyllin, respectively). In conclusion, *S. platensis* and its tetrapyrrolic components substantially decreased the proliferation of experimental pancreatic cancer. These data support a chemopreventive role of this edible alga. Furthermore, it seems that dietary supplementation with this alga might enhance systemic pool of tetrapyrroles, known to be higher in subjects with Gilbert syndrome.

Koníčková R. and al. Anti-cancer effects of blue-green alga Spirulina platensis, a natural source of bilirubin-like tetrapyrrolic compounds. Ann Hepatol. 2014 Mar-Apr;13(2):273-83.

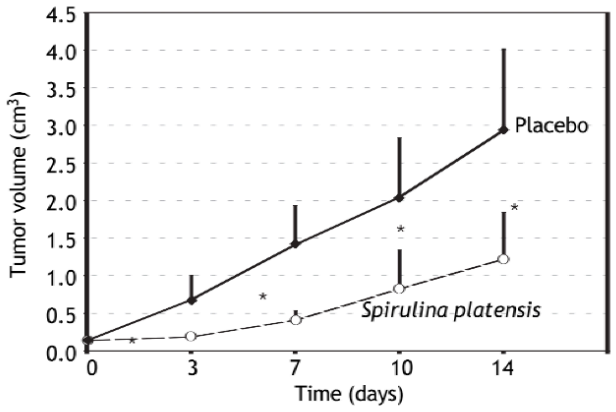


Figure 7. In vivo *anticancer effects of* S. platensis. *Athymic nu/nu mice xenotransplanted with PA-TU-8902 pancreatic cancer cells received placebo (water) or water suspension of freeze-dried* S. platensis *(0.5 g·kg⁻¹) intragastrically. Data expressed as mean ± SD. *p < 0.01.*

In vivo* anticancer effects of *S. platensis

Romay C, Armesto J, Remirez D, Gonzalez R, Ledon L, Garcia I. Antioxidant and anti-inflammatory properties of c-phycocyanin from blue-green algae. Inflamm Res. 1998;47:36-41.

In this study, Md. Ismail et al. evaluated the effect of spirulina intervention on oxidative stress, antioxidant status, and lipid profile of chronic obstructive pulmonary disease (COPD) patients. The serum content of malondialdehyde (MDA), lipid hydroperoxide, glutathione (GSH), vitamin C, cholesterol, triglyceride (TG), and high density lipoprotein (HDL) was measured. The activity of superoxide dismutase (SOD), catalase (CAT), and glutathione-s-transferase (GST) was also measured. Two different doses, (500 × 2) mg and (500 × 4) mg spirulina, were given to two groups of 15 patients. All targeted blood parameters have significant difference (P = 0.000) between COPD patients and controls except triglyceride (TG). Spirulina intake for 30 and 60 days at (500 × 2) mg dose has significantly reduced serum content of MDA, lipid hydroperoxide, and cholesterol (P = 0.000) while increasing GSH, Vit C level (P = 0.000), and the activity of SOD (P = 0.000) and GST (P = 0.038). At the same time, spirulina intake for 30 and 60 days at (500 × 4) mg dose has favorable significant effect (P = 0.000) on all targeted blood parameters except for HDL (P = 0.163)

Md. Ismail et al.. Effect of Spirulina Intervention on Oxidative Stress, Antioxidant Status, and Lipid Profile in Chronic Obstructive Pulmonary Disease Patients. BioMed Research International. Volume 2015 (2015), Article ID 486120.

4.4 Anti-Cancer properties

Cancer is a complex disease that involves a sequence of gene-environment interactions in a progressive process that cannot occur without dysfunction in multiple systems, including DNA repair, apoptotic and immune functions. Cancer causing agents called carcinogens transform a normal cell to tumor or cancerous cell. Life style, food habits, environmental factors and hereditary mutations plays major role in carcinogenesis.

Doll and Peto were the fist to establish that 35% of all human cancer deaths appear to be associated with diet and nutrition. (*Doll R, Peto R. The causes of cancer: quantitative estimates of avoidable risks of cancer in the United States today. J Natl Cancer Inst. 1981; 66:1192-1308*). Since then numerous experimental, epidemiological, and clinical studies have proved this connection. There is also a recent body of evidence to suggest that physiological aging of the immune system may affect cell-mediated immunity that in turn results in cancer development, autoimmune disease, and susceptibility to infection. (*Clifford CK. Cancer and nutrition. In: Gershwin et al., eds. Nutrition and Immunology. New Jersey: Humana Press; 2000:375-388*).

Several studies show that Spirulina may offer some protection against certain forms of cancer through its effect on the immune system, through a direct effect in the repair of DNA, and antioxidant protection from reactive oxygen species generated during normal or abnormal metabolism and from toxic substances in the environment.

Clinical studies

Mathew et al., studied the effect of Spirulina on oral leukoplakia (a pre-cancerous lesion) in pan tobacco chewers in Kerala, India. In a study involving 44 subjects in the intervention group and 43 in the placebo group, they found that supplementation with Spirulina at 1 g/day for 1 year resulted in complete regression of lesions in 45% of the intervention group and 7% in the control group.

B. Mathew, R. Sankaranarayanan, P. P. Nair et al., "Evaluation of chemoprevention of oral cancer with Spirulina fusiformis," Nutrition and Cancer, vol. 24, no. 2, pp. 197–202, 1995.

In another study Schwartz et al. showed that algae-derived phycocyanin had a cytostatic and cytotoxic activity against squamous cell carcinoma (human or hamster)

Schwartz J, Troxler RF, Saffer BG. Algae-derived phycocyanin is both cytostatic and cytotoxic to oral squamous cell carcinoma (human or hamster). J Dent Res. 1987;66:160.

Mishima et al. have demonstrated inhibition of tumor invasion and metastasis by calcium spirulan (Ca-SP), a polysaccharide isolated from Spirulina platensis.

Mishima T, Murata J, Toyoshima M, Fujii K, Nakajima M, Hayashi T, Kato T, Saiki I. Inhibition of tumor invasion and metastasis by calcium spirulan (Ca-SP), a novel sulfated polysaccharide derived from a blue-green alga, Spirulina platensis. Clin Exp Metastasis.1998;16:541-550.

Koníčková R. and al. have demonstrated that Spirulina platensis and its tetrapyrrolic components (phycocyanobilin and chlorophyllin) substantially decreased the proliferation of experimental pancreatic cancer compared to untreated cells, in a dose-dependent manner, from 0.16 g•L-1 (S. platensis), 60 μM (phycocyanobilin), and 125 μM (chlorophyllin), $p<0.05$). The anti-proliferative effects of S. platensis were also shown in vivo, where inhibition of pancreatic cancer growth was evidenced since the third day of treatment ($p < 0.05$).

Antiviral properties of Spirulina platensis

In vitro antiviral and antimicrobial activities of Spirulina platensis, Mona Hetta et al., World Journal of Pharmacy and Pharmaceutical Sciences, Volume 3, Issue 6, p. 31--39, 2014.

Table 2: Antiviral effect of non toxic doses from *Spirulina platensis*.

Tested virus	Initial Viral titre	Final viral titre	% of reduction	Mean % of reduction
Adenovirus type 7*	$1X10^5$	$4X10^4$	60%	53.3%
	$1X10^6$	$5X10^5$	50%	
	$1x10^7$	$5x10^6$	50%	
Coxsackievirus B4*	$1X10^5$	$4X10^4$	60%	66.7%
	$1X10^6$	$4X10^5$	60%	
	$1x10^7$	$3x10^6$	70%	
Astrovirus type 1**	$1X10^5$	$2X10^4$	80%	76.7%
	$1X10^6$	$2X10^5$	80%	
	$1x10^7$	$3x10^6$	70%	
Rotavirus Wa strain*	$1X10^5$	$4X10^4$	60%	56.7%
	$1X10^6$	$4X10^5$	60%	
	$1x10^7$	$5x10^6$	50%	
Adenovirus type 40***	$1X10^4$	$5X10^3$	50%	50%
	$1X10^5$	$5X10^4$	50%	
	$1x10^6$	$5x10^5$	50%	

* Initial and final viral titre values are expressed as PFU/ml.
** Initial and final viral titre values are expressed as CC-RT-PCR units/ml
*** Initial and final viral titre values are expressed as CC-PCR units/ml

The combined F E′ 5–F E′ 8 fraction showed a remarkable activity against HSV- 1 with an IC 50 value of 6.8 µg/ml, comparable to the reference drug, acyclovir, which has a IC 50 value of 1.5 µg/ml.

Nattayaporn Chirasuwan et al. Anti HSV-1 activity of sulphoquinovosyl diacylglycerol isolated from Spirulina platensis. ScienceAsia 35 (2009): 137–141.

Table 1 Antiviral activity of Sephadex LH-20 column chromatography fractions and re-chromatographed fractions of EtOAc extract of *S. platensis*.

1st chromatog. fractions		2nd chromatog. fractions	
Fraction	Anti HSV-1 IC_{50} (µg/ml)	Fraction	Anti HSV-1 IC_{50} (µg/ml)
F_E3	36.80 ± 3.21	$F_{E'}3$	36.61 ± 3.86
F_E4	30.83 ± 3.42	$F_{E'}4$	15.82 ± 1.87
F_E5	39.35 ± 2.81	$F_{E'}5$	7.71 ± 1.44
F_E6	7.59 ± 1.21	$F_{E'}6$	2.42 ± 1.01
F_E7	3.01 ± 0.84	$F_{E'}7$	0.97 ± 0.36
F_E8	0.84 ± 0.31	$F_{E'}8$	4.35 ± 0.21
F_E9	1.55 ± 0.41	$F_{E'}9$	24.31 ± 2.07
F_E10	18.31 ± 2.07	$F_{E'}10$	42.80 ± 3.92
		$F_{E'}11$	37.30 ± 2.54

Values are expressed as mean $\pm$ SD ($n = 3$).

Koníčková R. and al. Anti-cancer effects of blue-green alga Spirulina platensis, a natural source of bilirubin-like tetrapyrrolic compounds. Ann Hepatol. 2014 Mar-Apr;13(2):273-83.

4.5 Anti-viral properties

Several constituents of Spirulina have been documented to have anti-viral properties: phycocyanin, calcium spirulan (Ca-SP) (a sulfated polysaccharide), gamma-linolenic acid (GLA) and sulfolipids such as sulphoquinovosyl diacylglycerol (SQDG).

Clinical studies

In this study, the effect of calcium spirulan (Ca-SP) on anti-human immunodeficiency virus type 1 (HIV-1) and anti-herpes simplex virus type 1 (HSV-1) was tested. The results showed that Ca-SP is a potent antiviral agent against both HIV-1 and HSV-1. Furthermore, Ca-SP is quite promising as an anti-HIV agent because even at low concentrations of Ca-SP an enhancement of virus-induced syncytium formation was not observed, as was observed in dextran sulfate (DS) treated cultures, Ca-SP had very low anticoagulant activity, and showed a much longer half-life in the blood of mice when compared with that of DS.

Hayashi K, Hayashi T, Kojima I. A natural sulfated polysaccharide, calcium spirulan, isolated from Spirulina platensis: in vitro and ex vivo evaluation of anti-herpes simplex virus and anti-human immunodeficiency virus activities. AIDS Res Hum Retroviruses. 1996 Oct 10;12(15):1463-71.

Loya and al. showed that the 2-5% of sulfolipids contained in spirulina are effective against human immunodeficiency virus by selectively acting against DNA polymerase.

Loya, S. et al. The inhibition of the reverse transcriptase of HIV-1 by the natural sulfoglycolipids from cyanobacteria: contribution of different moieties to their high potency. J. Nat. Prod.,1998, 61(7), 891-5

Hernández-Corona A. and al. demonstrated the antiviral activity of a hot water extract (HWE) from Spirulina using several viruses. The HWE inhibited the infection for: herpes simplex virus type 2 (HSV-2), pseudorabies virus (PRV), human cytomegalovirus (HCMV), and HSV-1. The 50% effective inhibition doses (ED(50)) were 0.069, 0.103, 0.142, and 0.333 mg/ml for each virus, respectively.

Hernández-Corona A. and al. Antiviral activity of Spirulina maxima against herpes simplex virus type 2. Antiviral Research Volume 56, Issue 3, December 2002, Pages 279–285

Kaushik et al. showed that addition of allophycocyanin to the cells before viral infection has a great impact on preventing enterovirus infection due to interfering with adsorption and penetration of the virus.

Shih, S.R., Tsai, K.N., Li, Y.S., Chueh, C.C., Chan, H.C. Inhibition of enterovirus 71-induced apoptosis by allophycocyanin isolated from a blue-green alga Spirulina platensis, J. Med. Virol., 2003, 70(1), 119-25.

Rahman and al. showed that calcium spirulan can reduce viral replication by 50%.

Rahman, M.M., Escobedo-Bonilla C.M., Wille, M., Alday Sanz, V., Audoorn, L., Neyts, J., Pensaert, M.B., Sorgeloos, P., Nauwynck, H.J. Clinical effect of cidofovir and a diet supplemented with Spirulina platensis in white spot syndrome virus (WSSV) infected specific pathogen-free Litopenaeus vannamei juveniles. Aquaculture., 2006, 255, 600-605.

In this study, sulphoquinovosyl diacylglycerol (SQDG) was identified as the active antiviral agent. The results showed that the compound exhibited a remarkable activity against HSV-1 with an IC 50 value of 6.8 µg/ml, which was comparable to that of acyclovir (1.5 µg/ml). Based on previous studies. According to previous studies SQDG could have inhibitory effects on DNA polymerase, HIV-reverse transcriptase, P-selectin receptors and telomerase.

Nattayaporn Chirasuwan et al. Anti HSV-1 activity of sulphoquinovosyl diacylglycerol isolated from Spirulina platensis. ScienceAsia 35 (2009): 137–141

Antibacterial properties of Spirulina platensis

In vitro antiviral and antimicrobial activities of Spirulina platensis, Mona Hetta et al., World Journal of Pharmacy and Pharmaceutical Sciences, Volume 3, Issue 6, p. 31--39, 2014.

Table 3. Antimicrobial activity of *n*-hexane extract against *Spirulina platensis*

Bacterial strains	Control	Strains + *Spirulina platensis* extract	% Inhibition
Escherichia coli (ATCC 25922)	1.6×10^3	1×10^3	40.4
Salmonella typhimurium (ATCC 14028)	1.38×10^3	6.6×10^2	52.1
Salmonella senftenberg (ATCC 43845)	2.46×10^3	1×10^3	58.5
Candida albicans (ATCC 10231)	1.32×10^3	6×10^3	54.5
Listeria monocytogenes (ATCC 25152)	2.2×10^2	1.26×10^2	42.7
Pseudomonas aeruginosa (ATCC 15442)	1.1×10^3	4.8×10^2	55.5
**Enterococcus faecalis*	3.1×10	1.5×10	51.6
Staphylococcus aureus (ATCC 6538)	1.7×10^3	9.6×10^2	44.8
**Helicobacter pylori*	2.6×10^3	1.86×10^3	29.5

*local strain (Culture Collection of Bacteriological Lab, Water Pollution Research Department, National Research Center)

Therapeutic utility of Spirulina in Diabetes Mellitus

From Therapeutic utility of Spirulina, Uliyar V. Mani et al. Spirulina in human nutrition and health, Gerswhin M E and Belay A, CRC Press, 2008

TABLE 4.2
Effect of *Spirulina* Supplementation on Blood Glucose, HbA_{1c}, Glucosamine, and Uronic Acid Levels of Diabetic Patients (Mean ± SD)

Variables	0 months	2 months	4 months	*F* value
FBG				
Total patients ($n = 30$)	168.0 ± 52.2	150.2 ± 39.3*	137.7 ± 44.0***	8.20***
Males ($n = 17$)	168.7 ± 57.1	149.6 ± 35.5	138.6 ± 39.2*	3.05
Females ($n = 13$)	167.2 ± 47.3	150.9 ± 45.2	136.5 ± 51.4**#	9.23**
PP_2BG				
Total patients ($n = 30$)	262.0 ± 57.6	245.5 ± 66.4**	209.6 ± 50.3***	10.43***
Males ($n = 17$)	269.4 ± 60.6	251.1 ± 64.3	212.2 ± 43.1**#	7.10**
Females ($n = 13$)	252.3 ± 45.4	238.1 ± 70.9	206.2 ± 60.0*	3.23
HbA_{1c}				
Total patients ($n = 30$)	8.8 ± 1.8	8.2 ± 1.3*	8.1 ± 1.5*	
Males ($n = 17$)	8.5 ± 1.2	8.2 ± 0.9	8.1 ± 0.8*	
Females ($n = 13$)	8.4 ± 1.2	7.8 ± 1.3*	7.4 ± 1.3*	
Glucosamine				
Total patients ($n = 30$)	4.3 ± 1.3	3.9 ± 1.3**	3.8 ± 1.4**	5.65**
Males ($n = 17$)	4.5 ± 1.4	4.2 ± 1.3*	4.1 ± 1.2*	3.58*
Females ($n = 13$)	3.9 ± 1.3	3.6 ± 1.3*	3.5 ± 1.7*	2.07
Uronic acid				
Total patients ($n = 30$)	50.7 ± 10.3	44.8 ± 10.3***	43.2 ± 11.2***	15.46***
Males ($n = 17$)	53.4 ± 11.3	47.5 ± 10.3***	46.9 ± 9.9**	10.70***
Females ($n = 13$)	47.2 ± 8.0	41.3 ± 9.1*	38.4 ± 11.4*	5.84**

ANOVA: $^{**}p < .01$, $^{***}p < .001$.
$^{*}p < .05$, $^{**}p < .01$, $^{***}p < .001$ vs. 0 months.
$^{\#}p < .05$, $^{\#\#}p < .01$, $^{\#\#\#}p < .001$ vs. 2 months.

4.6 Antibacterial properties

According to Demule et al., the antibacterial activity of Spirulina is due to the presence of gamma-linolenic acid, an antibiotically-active fatty acid present in a high concentration in this alga. (Demule, M.C.Z., Decaire, G.Z., Decano, M.S. Bioactive substances from Spirulina platensis (cianobacteria). Int. J. Exp. Bot., 1996, 58, 93-96.)

Clinical studies

This in vitro study showed tha Spirulina extract has a positive effect on the inhibition of serveral bacterias : Gram-positive bactria (Staphylococcus aureus) and Gram-negative bacteria (Escherichia coli , Pseudomonas aeruginoas and Salmonella typhi).

Kaushik P., Chauhan Abhishek. In vitro antibacterial activity of laboratory grown culture of Spirulina platensis. Indian J. Microbiol. September 2008. 48:348–352

Mendiola et al. studied the antimicrobial activities of Spirulina extract against Staphylococcus aureus (gram positive bacterium), Escherichia coli (gram negative bacterium), Candida albicans (yeast) and Aspergillus niger (fungus). Results showed that C. albicans were the most sensitive microorganism to all Spirulina fractions.

Mendiola, J.A., Jaime, L., Santoyo, S., Reglero, G., Cifuentes, A., Ibanez, E., Senorans, F.J. Screening of functional compounds in supercritical fluid extracts from Spirulina platensis. Food chem., 2007, 102, 1357-67.

4.7 Digestive properties

Spirulina possesses prebiotic properties and stimulates the production of lactobacillus. Lactobacillus play an important role, they improve our digestion and absorption of foods, protect us from infection and stimulate our immune system.

Clinical studies

This study showed that an intake of Spirulina at 5% of the diet increased the population of Lactobacillus in the caecum of rats by 327 % over a control group of rats not fed with spirulina.

Tsuchihashi et al. Effect of Spirulina platensis on caecum content in rats. Bull Chiba Hygiene College. 1987;5:27-30.

This study demonstrated the stimulatory effect of spirulina on lactic acid bacteria, including Lactococcus lactis, Streptococcus thermophilus, Lactobacillus casei, Lactobacillus acidophilus and Lactobacillus bulgaricus.

Parada JL et al. Lactic acid bacteria growth promoters from Spirulina platensis. Int J Food Microbiol. 1998;45:225-228.

4.8 Anti-diabetes properties (blood sugar level and insulin resistance lowering properties)

The antidiabetic properties of Spirulina are due to its gamma-linolenic-acid, antioxidants, amino and fatty acids, superoxide dismutase and phycocyanin. They are able to reduce significantily the blood sugar and cholosterol levels. Phycocyanin seems to enhance the insulin sensitivity and to regulate the metabolism of glucolipides.

Clinical studies

In this study, oral administration of Phycocyanin (100 mg/kg, once per day for 3 weeks) on KKAy mice was investigated. The results showed that the administration of phycocyanin significantly decreased the body weight, fasting plasma glucose, 24 h random blood glucose levels, FINS and GSP levels, TG and TC content in serum and livers, MDA content in livers ($p < 0.05$ or $p < 0.01$). On the other hand, glucose tolerance to glucose administration, T-AOC, and the content of glycogen in liver and muscle were enhanced ($p < 0.05$ or $p < 0.01$). Histopathological results showed that Phycocyanin administration suppressed the abnormal enlargement of islets observed in the pancreas of KKAy mice.

Ou Y, Lin L, Yang X, Pan Q, Cheng X. Antidiabetic potential of phycocyanin: effects on KKAy mice. Pharm Biol. 2013 May;51(5):539-44. doi: 10.3109/13880209.2012.747545. Epub 2013 Feb 1.

This study aimed to evaluate the hypoglycemic and hypolipidemic role of Spirulina. Twenty-five subjects with type 2 diabetes mellitus were randomly assigned to receive Spirulina (study group) or to form the control group. The efficacy of Spirulina supplementation was determined with a dosis of 2 g/day for 2 months. The results showed: an

Therapeutic utility of Spirulina in Diabetes Mellitus

From Therapeutic utility of Spirulina, Uliyar V. Mani et al. Spirulina in human nutrition and health, Gerswhin M E and Belay A, CRC Press, 2008

TABLE 4.3
Effect of *Spirulina* Supplementation on Lipids and Lipoprotein Levels of Diabetic Patients (Mean ± SD)

Variables	0 months	2 months	4 months	*F* value
Triglycerides				
Total patients ($n = 30$)	160.8 ± 62.4	149.5 ± 78.8	132.7 ± 53.0$^{**\#}$	5.18**
Males ($n = 17$)	155.9 ± 79.8	150.1 ± 81.8	137.8 ± 53.5	1.56
Females ($n = 13$)	151.3 ± 52.8	132.5 ± 50.7	116.8 ± 39.6$^{*\#}$	6.85**
Total lipids				
Total patients ($n = 30$)	648.9 ± 166.4	618.6 ± 157.1	582.9 ± 145.4$^{**\#}$	5.86**
Males ($n = 17$)	652.1 ± 179.5	640.8 ± 162.8	604.2 ± 153.4	1.46
Females ($n = 13$)	644.6 ± 154.8	589.6 ± 150.7	555.1 ± 135.1$^{**\#}$	7.81**
TC				
Total patients ($n = 30$)	198.5 ± 37.2	190.6 ± 31.7	183.4 ± 25.7$^{***\#}$	6.62**
Males ($n = 17$)	189.5 ± 38.6	186.4 ± 34.4	184.9 ± 29.2	0.47
Females ($n = 13$)	210.2 ± 33.0	196.2 ± 28.0	181.4 ± 21.2$^{**\#\#\#}$	10.32***
HDL-C				
Total patients ($n = 30$)	43.6 ± 13.0	44.4 ± 11.4	48.4 ± 12.2$^{***\#\#}$	8.01***
Males ($n = 17$)	39.8 ± 8.1	40.0 ± 7.5	44.2 ± 8.7$^{**\#\#}$	1.46
Females ($n = 13$)	48.4 ± 17.0	50.4 ± 13.1	53.8 ± 14.4	2.28
LDL-C				
Total patients ($n = 30$)	123.9 ± 28.4	116.4 ± 23.9*	108.3 ± 21.0$^{**\#\#}$	10.25***
Males ($n = 17$)	118.0 ± 30.5	114.1 ± 25.7	111.6 ± 23.6	1.63
Females ($n = 13$)	131.6 ± 24.4	119.4 ± 22.0	103.9 ± 17.0$^{**\#\#}$	11.60***
VLDL-C				
Total patients ($n = 30$)	30.9 ± 13.4	29.9 ± 15.7	27.1 ± 10.5*	2.54
Males ($n = 17$)	32.5 ± 18.8	31.5 ± 15.5	29.0 ± 11.9	0.94
Females ($n = 13$)	30.2 ± 10.6	26.5 ± 10.0	23.5 ± 7.5$^{*\#}$	6.42**
Non HDL-C				
Total patients ($n = 30$)	154.8 ± 33.4	146.4 ± 29.2*	135.0 ± 25.8$^{***\#\#\#}$	13.55***
Males ($n = 17$)	149.5 ± 37.6	146.8 ± 34.2	140.6 ± 28.8	1.92
Females ($n = 13$)	161.8 ± 26.7	145.8 ± 22.4*	127.6 ± 16.6$^{***\#\#\#}$	20.17***
TC:HDL-C				
Total patients ($n = 30$)	4.8 ± 1.1	4.5 ± 1.1	3.9 ± 1.1$^{***\#\#\#}$	16.51***
Males ($n = 17$)	4.8 ± 1.2	4.7 ± 1.2	4.3 ± 1.0$^{**\#}$	5.55**
Females ($n = 13$)	4.7 ± 1.1	4.1 ± 0.9**	3.6 ± 0.8$^{***\#\#}$	21.78***
LDL-C:HDL-C				
Total patients ($n = 30$)	3.0 ± 0.9	2.5 ± 0.9**	2.4 ± 0.7***	11.61***
Males ($n = 17$)	3.0 ± 0.9	2.8 ± 0.7	2.6 ± 0.8*	3.81*
Females ($n = 13$)	3.0 ± 0.9	2.5 ± 0.6*	2.0 ± 0.6$^{***\#\#\#}$	21.34***

ANOVA: $^{*}p < .05$, $^{**}p < .01$, $^{***}p < .001$.
$^{*}p < .05$, $^{**}p < .01$, $^{***}p < .001$ vs. 0 months.
$^{\#}p < .05$, $^{\#\#}p < .01$, $^{\#\#\#}p < .001$ vs. 2 months.

appreciable lowering of fasting blood glucose and postprandial blood glucose levels.; a significant reduction in the HbA(1c) level, indicating improved long-term glucose regulation; a significant lowering of lipids, triglyceride levels. Total cholesterol (TC) and its fraction, low-density lipoprotein cholesterol (LDL-C), exhibited a fall coupled with a marginal increase in the level of high-density lipoprotein cholesterol (HDL-C). As a result, a significant reduction in the atherogenic indices, TC: HDL-C and LDL-C: HDL-C, was observed. The level of apolipoprotein B registered a significant fall together with a significant increment in the level of apolipoprotein A1. Therefore, a significant and favorable increase in the ratio of A1:B was also noted. These findings suggest the beneficial effect of Spirulina supplementation in controlling blood glucose levels and in improving the lipid profile of subjects with type 2 diabetes mellitus.

Parikh P, Mani U, Iyer U. Role of Spirulina in the Control of Glycemia and Lipidemia in Type 2 Diabetes Mellitus. J Med Food. 2001 Winter;4(4):193-199.

The following study was made with 160 non insulin dependent diabetics in order to determine the effect of supplementation of Spirulina on fasting blood glucose, fasting glycosylated haemoglobin and lipid profile. on diet, drugs and insulin. A daily dosis of 1g was given during 90 days. There was a statistically significant reduction ($P < 0.001$) from pre to post levels of fasting blood glucose, glycosylated hemoglobin and lipid profile levels of the diabetics. There was an increase in HDL – cholesterol levels from pre to post. The conclusion of the study was: "From the study it can be concluded that Spirulina is a potent neutraceutical both as a hypoglycemic and hypolipidemic agent in NIDDM volunteers".

L. Anitha and K. Chandralekha. Effect of Supplementation of Spirulina on Blood Glucose, Glycosylated Hemoglobin and Lipid Profile of Male N. on Insulin Dependent Diabetics. ASIAN J. EXP. BIOL. SCI., VOL 1 (1) 2010:36-46

4.9 Cholesterol lowering properties

Collectively the results of different animal and human studies provide support for the cholesterol-lowering activity of Spirulina. A vast amount of experimental and epidemiological evidence shows the connection between diets high in fat and cholesterol and the incidence of cardiovascular disease.

There is also an increased awareness among Americans that diets high in cholesterol present a risk of cardiovascular disease. Despite this, cardiovascular disease is the number one killer in the United States, claiming about one million lives a year and totaling 41% of all deaths. It is often said that a fast lifestyle makes it difficult for many Americans to make proper food choices. Supplementation with natural food supplements like Spirulina may contribute, in part at least, to an overall strategy to manage this serious health problem

Clinical studies

Nakaya et al. [17], in the first human study, gave 4.2 g day $^{-1}$ of Spirulina to 15 male volunteers and, although there was no significant increase in high-density lipoprotein (HDL) levels, they observed a significant reduction of high-density lipoprotein (LDL) cholesterol after 8 weeks of treatment. The atherogenic e ff ect also declined significantly in the above group [17].

N. Nakaya, Y. Homa, and Y. Goto, "Cholesterol lowering effect of Spirulina," Atherosclerosis, vol. 37, pp. 1329–1337, 1988.

Ramamoorthy and Premakumari [18] in a more recent study administered Spirulina supplements in ischemic heart disease patients and found a significant reduction in blood cholesterol, triglycerides and LDL cholesterol and an increase in HDL cholesterol.

A. Ramamoorthy and S. Premakumari, "E ff ect of supplementation of Spirulina on hypercholesterolemic patients," Journal of Food Science and Technology, vol. 33, no. 2, pp. 124–128, 1996.

Elias E. Mazokopakis et al. showed that, at the end of the 6-month intervention period, the mean levels of triglycerides, low-density lipoprotein-cholesterol, total cholesterol, and the ratio of total cholesterol to high-density lipoprotein cholesterol were significantly decreased, whereas the mean levels of high-density lipoprotein-cholesterol and hemoglobin were significantly increased. Spirulina supplementation resulted also in a significant reduction in weight and HOMA-IR index (Insulin Resistance Index) and a significant improvement in health-related quality of life scale.

Elias E. Mazokopakis et al. The hepatoprotective and hypolipidemic effects of Spirulina (Arthrospira platensis) supplementation in a Cretan population with non-alcoholic fatty liver disease: a prospective pilot study. Annals of Gastroenterology (2014) 27, 387-394

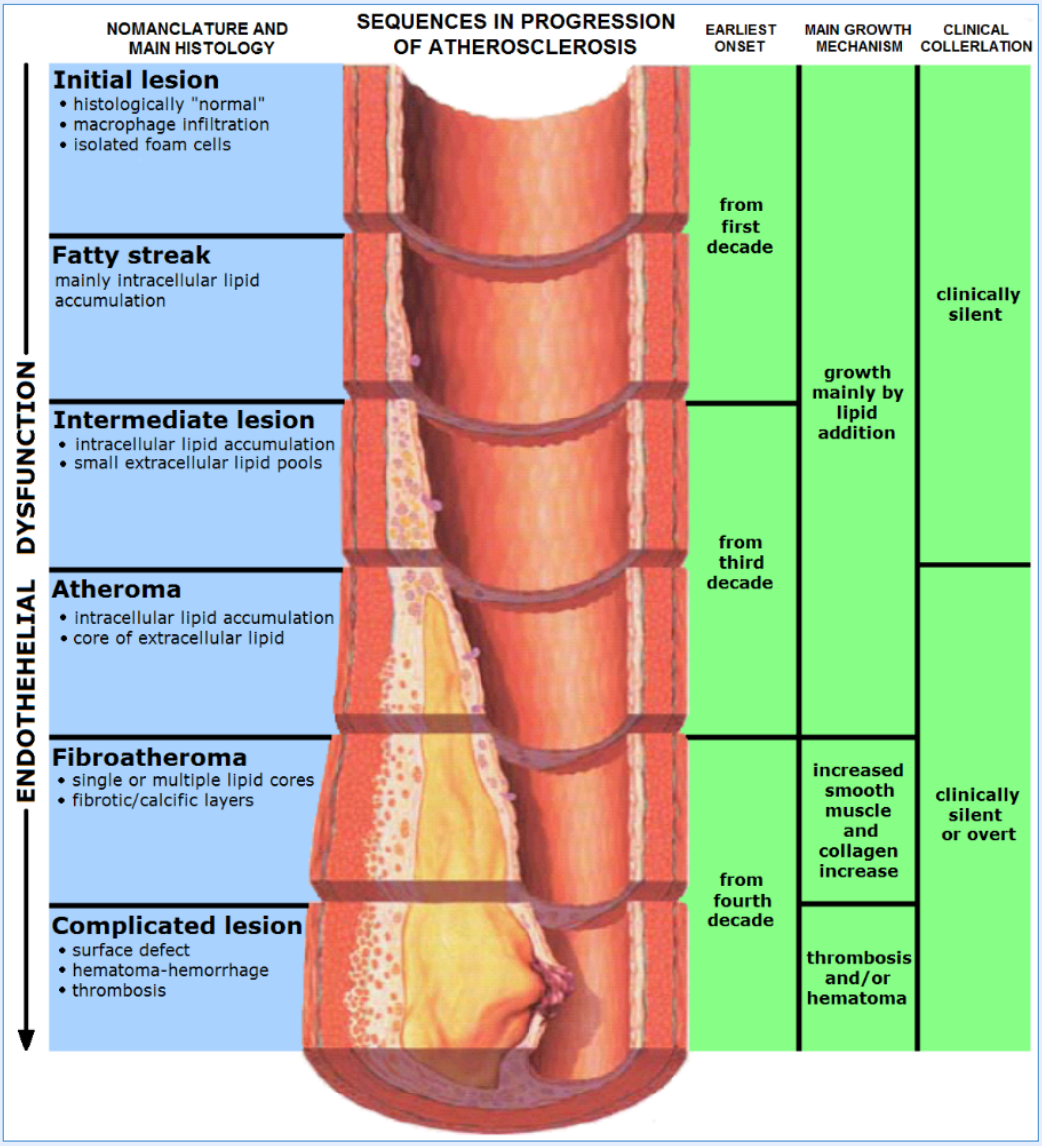
Lipids in Health and Disease 2007, **6**:33 http://www.lipidworld.com/content/6/1/33

Table 4: Initial and Final High Blood Pressure Prevalences* (%)

BP	Normal	Prehypertension	Hypertension Stage I	Hypertension Stage 2
Initial	11	44	31	14
Final	36	50	11	3

BP: blood pressure. * p = 0.01 initial *vs.* final, chi-squared test, n = 36.

Patricia V Torres-Duran et al. Antihyperlipemic and antihypertensive effects of Spirulina maxima in an open sample of mexican population: a preliminary report. Lipids in Health and Disease 2007, 6:33 doi:10.1186/1476-511X-6-33



4.10 Antihypertensive (high blood pressure lowering) properties

The antihypertensive properties of Spirulina are due its content of phycocyanin and ACE inhibitory peptide Ile-Gln-Pro.

Clinical studies

The antihypertensive effect of an angiotensin I-converting enzyme (ACE) inhibitory peptide Ile-Gln-Pro (IQP), whose sequence was derived from Spirulina platensis, was investigated in spontaneously hypertensive rats (SHRs) for 1 week. The weighted systolic blood pressure (SBP) and diastolic blood pressure (DBP) of the peptide IQP-treated group were significantly lower than those of the negative control group from the third and fourth days, respectively. Accompanying the blood pressure reduction, a significant regulation of the expression of major components of the renin-angiotensin system (RAS) was found in the treatment group, including downregulation of the mRNA levels of renin, ACE, and the angiotensin II type 1 (AT1) receptor in the kidney, as well as serum angiotensinogen (Ang), ACE, and angiotensin II (Ang II) concentrations. The treatment group also showed upregulation of mRNA expression of the angiotensin II type 2 (AT2) receptor in the kidney. Our findings suggested that IQP might be of potential use in the treatment of hypertension.

Lu J, Sawano Y, Miyakawa T, Xue YL, Cai MY, Egashira Y, Ren DF, Tanokura M. One-week antihypertensive effect of Ile-Gln-Pro in spontaneously hypertensive rats. J Agric Food Chem. 2011 Jan 26; 59(2): 559-63.

In this study, the researchers hypothesized that phycocyanin derived from Spirulina exerts antihypertensive actions by improving endothelial dysfunction in metabolic syndrome. Spontaneously hypertensive/NIH-corpulent (SHR/NDmcr-cp) rats were divided into 4 groups then fed a normal diet with or without phycocyanin (2500-, 5000-, or 10,000-mg/kg diet) for 25 weeks. At 34 weeks of age, although systolic blood pressure was not significantly different among groups, phycocyanin-fed groups exhibited a dose-dependent decrease in blood pressure. The conclusion was that phycocyanin may be beneficial for preventing endothelial dysfunction-related diseases in metabolic syndrome

Ichimura M et al. Phycocyanin prevents hypertension and low serum adiponectin level in a rat model of metabolic syndrome. Nutr Res. 2013 May;33(5):397-405.

The purpose of this study was to evaluate the effects of Spirulina maxima orally supplied (4.5 g/day, for 6 weeks) to a sample of 36 subjects (16 men and 20 women, with ages between 18–65 years). The volunteers did not modify their dietary habits or lifestyle during the whole experimental period. It showed that Spirulina reduced systolic and diastolic blood pressure in both male and female: SYST-P male 121 ± 9 vs. 111 ± 8 mm Hg ($p < 0.01$), DIAST-P male 85 ± 6.5 vs. 77 ± 9 mm Hg ($p < 0.01$); SYST-P female 120 ± 9.5 vs. 109 ± 11 mm Hg ($p < 0.002$), DIAST-P female 85 ± 11 vs. 79 ± 7.5 mm Hg ($p < 0.03$).

Patricia V Torres-Duran et al. Antihyperlipemic and antihypertensive effects of Spirulina maxima in an open sample of mexican population: a preliminary report. Lipids in Health and Disease 2007, 6:33 doi:10.1186/1476-511X-6-33

4.11 Protection against atherosclerosis

Although the exact cause is unknown, atherosclerosis may start with damage or injury to the inner layer of an artery caused by high blood pressure, high cholesterol, high triglycerides, smoking, diabetes or inflammation.

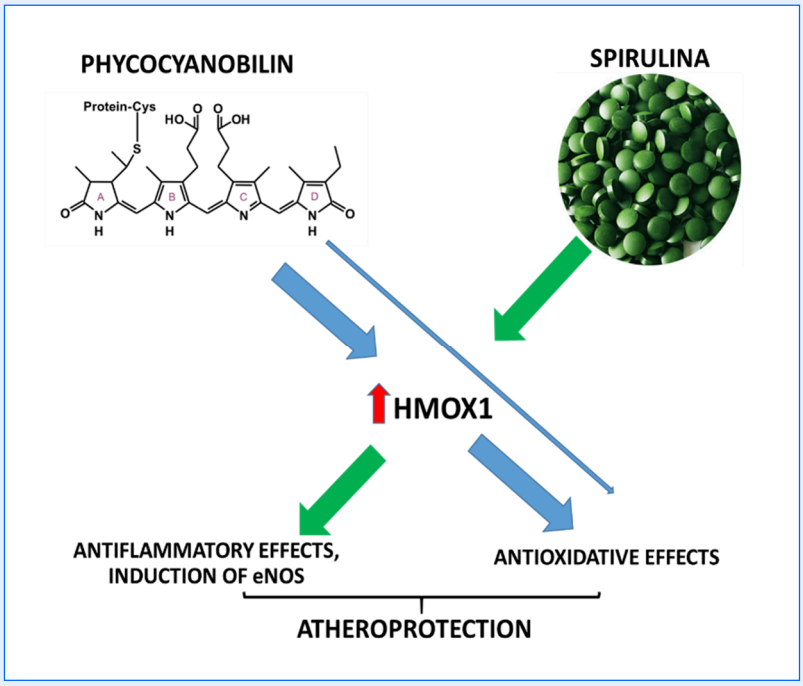
Once the inner wall of an artery is damaged, blood cells and other substances often clump at the injury site and build up in the inner lining of the artery. Over time, fatty deposits (plaques) made of cholesterol and other cellular products also build up at the injury site and harden, narrowing your arteries. The organs and tissues connected to the blocked arteries then don't receive enough blood to function properly. Eventually pieces of the fatty deposits may break off and enter your bloodstream. This may cause a blood clot, which can block the blood flow to a specific part of your body. A blood clot can also travel to other parts of your body, blocking flow to another organ. Atherosclerosis can lead to serious problems, including heart attack, stroke, or even death.

Clinical studies

The anti-atherogenic effects of spirulina (Spirulina platensis) were investigated in the New Zealand White (NZW) rabbit model. The animal had hypercholesterolemia induced by being fed a high cholesterol diet (HCD) containing 0.5% cholesterol for 4 weeks, and then fed a HCD supplemented with 1 or 5% spirulina (SP1 or SP5) for an additional

Atheroprotective Effect of Spirulina

Strasky Z et al.. Spirulina platensis and phycocyanobilin activate atheroprotective heme oxygenase-1: a possible implication for atherogenesis. Food Funct. 2013 Nov;4(11):1586-94.



Focus on

Heavy Metals

Heavy metals are individual metals and metal compounds that can impact human health. Eight common heavy metals are discussed in this brief: arsenic, barium, cadmium, chromium, lead, mercury, selenium, and silver. Although some individuals are primarily exposed to these contaminants in the workplace, for most people the main route of exposure to these toxic elements is through the diet (food and water).

Cadmium is a very toxic metal, known human *carcinogens*. **Smokers** are significantly exposed to high cadmium levels. Long-term exposure to lower levels leads to a buildup in the kidneys and possible kidney disease, lung damage, and fragile bones.

Mercury can be found in **fish** and **dental fillings**. The nervous system is very sensitive to all forms of mercury. Exposure to high levels can permanently damage the brain, kidneys, and developing fetuses. Effects on brain functioning may result in irritability, shyness, tremors, changes in vision or hearing, and memory problems.

Effective antagonists of a specific toxic metal will be a chelating agent which can gain access to the metal in vivo and which forms a very stable complex, of modest toxicity with that metal ion which is rapidly excreted without damage to the kidney or liver.

8 weeks. Spirulina supplementation lowered intimal surface of the aorta by 32.2 to 48.3%, compared to HCD. Serum triglyceride (TG) and total cholesterol (TC) significantly were reduced in SP groups. After 8 weeks, serum low density lipoprotein cholesterol (LDL-C) remarkably decreased by 26.4% in SP1 and 41.2% in SP5, compared to HCD. On the other hand, high density lipoprotein cholesterol (HDL-C) was markedly increased in SP1 and SP5 compared with that in the HCD group from 2 to 8 wk. These results suggest that spirulina intake can cause the reduction of hypercholesterolemic atherosclerosis, associated with a decrease in levels of serum TC, TG and LDL-C, and an elevation of HDL-C level. Spirulina may, therefore, be beneficial in preventing atherosclerosis and reducing risk factors for cardiovascular diseases.

Cheong SH et al. Spirulina prevents atherosclerosis by reducing hypercholesterolemia in rabbits fed a high-cholesterol diet. J Nutr Sci Vitaminol (Tokyo). 2010;56(1):34-40.

The study investigated whether S. platensis or phycocyanobilin (PCB), its tetrapyrrolic chromophore, can activate atheroprotective heme oxygenase-1 (Hmox1), a key enzyme in the heme catabolic pathway responsible for generation of a potent antioxidant bilirubin, in endothelial cells and in a mouse model of atherosclerosis. In vitro experiments were performed on EA.hy926 endothelial cells exposed to extracts of S. platensis or PCB. In vivo studies were performed on ApoE-deficient mice fed a cholesterol diet and S. platensis.
Both S. platensis and PCB markedly upregulated Hmox1 in vitro, and a substantial overexpression of Hmox1 was found in aortic atherosclerotic lesions of ApoE-deficient mice fed S. platensis. In addition, S. platensis treatment led to a significant increase in Hmox1 promoter activity in the spleens of Hmox-luc transgenic mice. Furthermore, both S. platensis and PCB were able to modulate important markers of oxidative stress and endothelial dysfunction, such as eNOS, p22 NADPH oxidase subunit, and/or VCAM-1. Both S. platensis and PCB activate atheroprotective HMOX1 in endothelial cells and S. platensis increased the expression of Hmox1 in aortic atherosclerotic lesions in ApoE-deficient mice, and also in Hmox-luc transgenic mice beyond the lipid lowering effect.

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4.12 Protection against heavy metals

The detoxification properties of Spirulina have been demonstrated in numerous studies and can be, among other reasons, attributed to its metal-binding capacities and related to its contents of vitamins E and C, beta-carotene, as well as enzyme superoxide dismutase (SOD), selenium and phycocyanin.

Clinical studies

According to Yamane et al., rats with high mercury dosage showed rising blood urea nitrogen (BUN) and serum creatinine, both indicators of acute nephritis. The addition of 30% Spirulina in the diet resulted in a significant decrease in BUN and serum creatinine levels.

Fukino H, Takagi Y, Yamane Y. Effect of Spirulina (S platensis) on the renal toxicity induced by inorganic mercury andcisplatin. Eisei Kagaku. 1990; 36:5.

Bermejo et al. demonstrated in this other *in vitro* study that S. platensis protein extract possessed an excellent antioxidant as well as an iron-chelating activity. Results showed that the protein extract of S. platensis scavenged hydroxyl and peroxyl radicals and also had inhibitory activity against lipid peroxidation. The iron ions decreased the maximum fluorescence emission spectra of S. platensis, which was an indicator of its metal-chelating activity. The conclusion was that the antioxidant properties of S. platensis and phycocyanin may arise from both its radical-scavenging and metal chelation properties.

Bermejo-Bescós, P., Piñero-Estrada, Villar, A.M. Iron-chelating ability and antioxidant properties of phycocyanin isolated from a protein extract of Spirulina platensis. Food. Chem., 2008, 110, 436-45.

The protective effect of S. platensis against cadmium-induced oxidative stress has been demonstrated in the following study:

Simsek, N., Karadeniz, A., Kalkan, Y., Keles, O.N., Unal, B. Spirulina platensis feeding inhibited the anemia- and leucopenia-induced lead and cadmium in rats. J. Hazard. Mater., 2009, 164, 1304-1039.

Antioxidative and Hepatoprotective Effects of Spirulina

Li-chen Wu, Ja-an Annie Ho, Spirulina in human nutrition and health, Gerswhin M E and Belay A, CRC Press, 2008

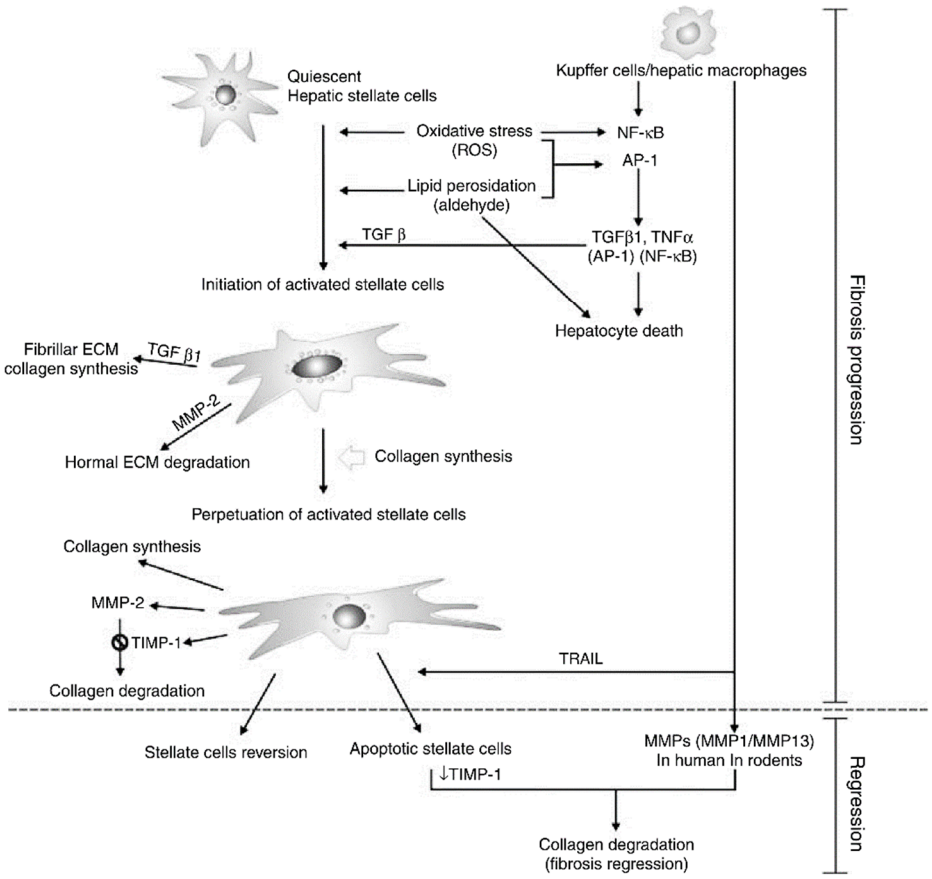


FIGURE 6.4 Summary of hepatic fibrosis and regression.

Antioxidative and Hepatoprotective Effects of Spirulina

Li-chen Wu, Ja-an Annie Ho, Spirulina in human nutrition and health, Gerswhin M E and Belay A, CRC Press, 2008

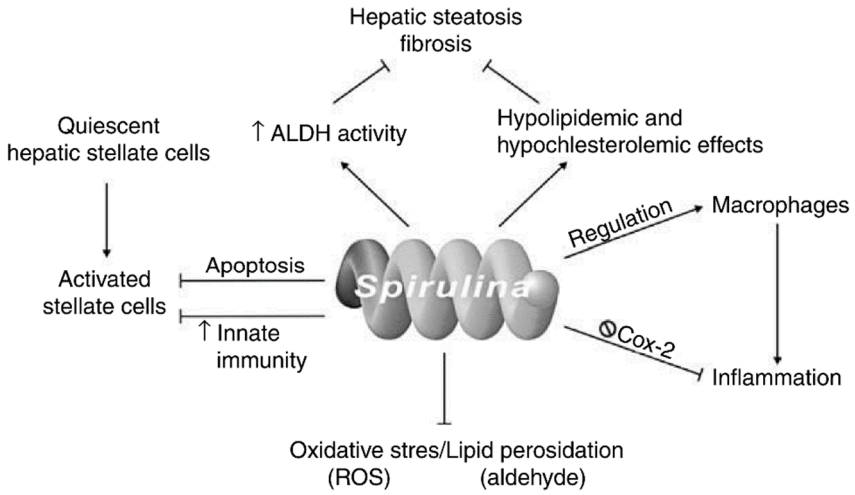


FIGURE 6.5 Effects of *Spirulina* on liver fibrosis.

The following studies showed that Spirulina improved the metabolism of iron and Hb in rats with Lead (Pb), Cadmium (Cd), Zinc (Zn), and Mercury (Hg) induced poisoning.

Simsek, N., Karadeniz, A., Kalkan, Y., Keles, O.N., Unal, B. Spirulina platensis feeding inhibited the anemia- and leucopenia-induced lead and cadmium in rats. J. Hazard. Mater., 2009, 164, 1304-1039.

Bermejo-Bescós, P., Piñero-Estrada, E., Villar del Fresno, A.M. Neuroprotection by Spirulina platensis protein extract and phycocyanin against iron-induced toxicity in SH-SY5Y neuroblastoma cells. Toxicol. In Vitro., 2008, 22, 1495-1502.

4.13 Hepatoprotective properties

Hepatoprotective properties of Spirulina derive mainly from:

- its antioxidant properties - Phycocyanin has been reported to be able to scavenge hydroxyl, alkoxyl, and peroxyl radicals induced by drugs or heavy metals. The free radical scavenging capacity reduces the generation of lipid peroxides, which disrupt the membrane structure and the biochemical functions of the liver. *See chapter "Antioxidant properties"*.
- its metalloprotective effects - Several studies have demonstrated that Spirulina possesses the metalloprotective effects. It is well established that heavy metals, such as lead and cadmium, impact the cellular growth, diminish cellular productivity, and induce toxicity in cells by accelerating iron dependent lipid peroxidation, ultimately leading to cellular death. *See chapter "Protection against heavy metals"*.
- Its effect on fatty liver - Fatty liver is a common cause of chronic liver disease and refers to accumulation of excess fat in the liver. It is often observed in alcoholics, obese persons, and diabetic patients. It is also frequently caused by drugs, viral hepatitis, chemical intoxication, pregnancy, intestinal bypass surgery and malnutrition. Considerable results suggest that Spirulina is able to control the formation of fatty liver trough its antioxidative and anti-inflammatory effects, induction of PGE2 production by GLA, hypolipidemic and hypochlesterolemic effects, and activated ALDH activity. *See also chapter "Cholesterol lowering properties"*.
- Its antifibrotic action - Hepatic fibrosis is a common outcome of the progressive accumulation of connective tissue in the liver in response to hepatocellular damage. The fibrotic process arises from excessive production of the extracellular matrix (ECM). Various cells and factors participate in fibrogenesis. Kupffer cells and HSC are mainly responsible for the fibrosis process. In addition, transforming growth factor β1 (TGF β 1) is essential for the fibrotic diseases. Liver fibrosis is usually associated with oxidative stress and its subsequent induced inflammation. Spirulina has been reportedly associated with the attenuation of fibrosis by the induction of HSC apoptosis and the antioxidative activity, which is involved in the reduction of oxidative stress. Spirulina can be used to attenuate fibrotic process through antioxidative effect, anti-inflammation, induction of apoptosis of HSC and probably enhanced innate immunity.

Clinical studies

Several studies have examined the use of C-phycocyanin in hepatoprotection. This protection derives mostly from its ability to scavenge reactive radicals, reducing hepatotoxicity.

Vadiraja, B.B., Gaikward, N.W., and Madyastha, K.M., Hepatoprotective effect of C-phycocyanin: protection for carbon tetrachloride and R-(+)-pulegone-mediated hepatotoxicity in rats, Biochem. Biophys. Res. Commun., 249, 428, 1998.

The hypolipidemic effects of Spirulina have been demonstrated in numerous studies, for instance:

Elias E. Mazokopakis et al. The hepatoprotective and hypolipidemic eff ects of Spirulina (Arthrospira platensis) supplementation in a Cretan population with non-alcoholic fatty liver disease: a prospective pilot study. Annals of Gastroenterology (2014) 27, 387-394

Spirulina has been reportedly associated with the attenuation of fibrosis by the induction of HSC apoptosis and the antioxidative activity, which is involved in the reduction of oxidative stress and a decrease in proinflammatory cytokine gene expression. In addition, C-phycocyanin reduces the extent of Kupffer cell phargocytosis.

Remirez, D. et al., Influence of C-phycocyanin on hepatocellular parameters related to liver oxidative stress and Kupffer cell functioning, Inflamm Res., 51, 351, 2002.

Spirulina, which contains many antioxidants such as phycocyanins, carotenoids, selenium, and some phenolics, suppresses oxidative stress and the up-regulation of proinflammatory cytokine expression. It may further attenuate the progress of liver fibrosis.

Parola, M. et al., Vitamin E dietary supplementation inhibits transforming growthfactor β 1 gene expression in the rat liver, FEBS Lett., 308, 267, 1992.

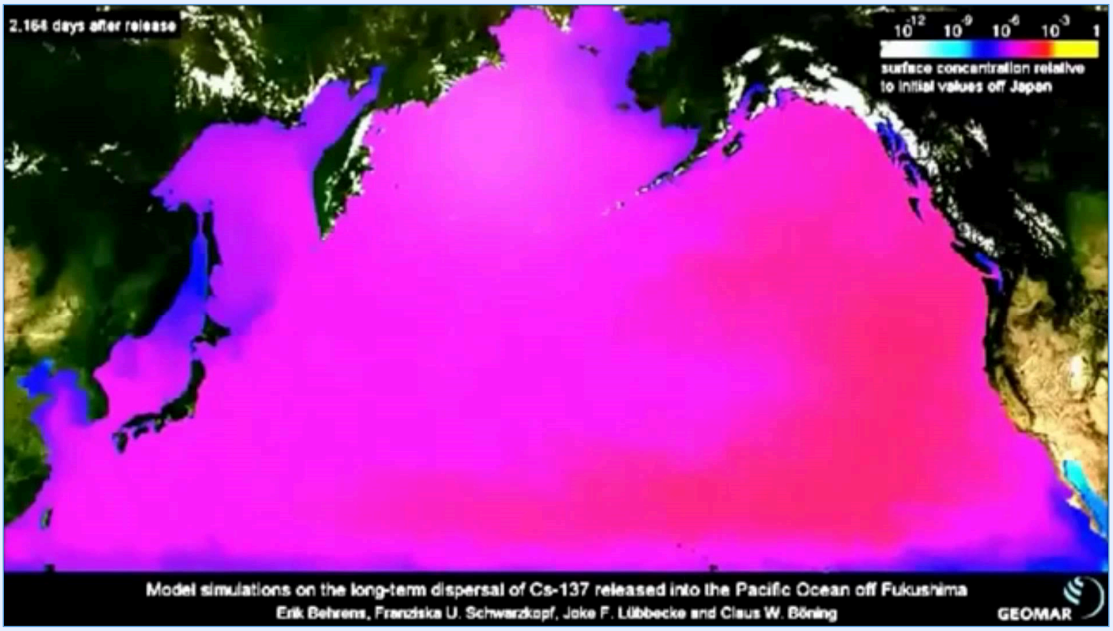
Focus on

Fukushima, radiations and seafood

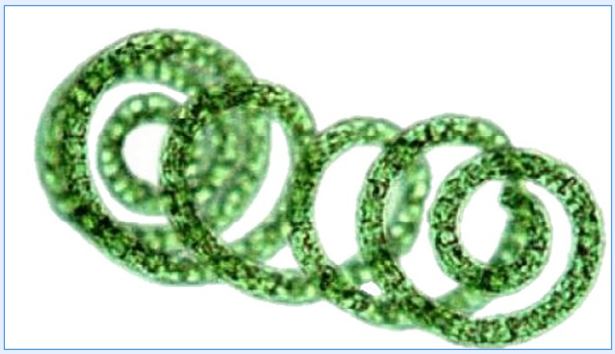
Because of Fukushima nuclear plant accident, Cesium-137 and Strontium-90, both radioactive, have been disseminated into the oceans, contaminating fishes and other seafoods.

According to the Scientific American, radioactivity has been detected in fishes near California. A raising numbe of mutations has also been observed.

Both radioactive strontium and cesium end up getting deposited in human bones, where the radiation wreaks havoc on bone marrow, causing bone cancers and blood cancers like Leukemia.



Fukushima radioactive pollution spreading into the oceans and poisoning seafood (Cesium-137 dispersion model – 10-year projection). Source: *organicslant.com.*



Microscopic view of Spirulina

4.14 Protection against radiation

DNA repair is a complex process involving enzymes and coenzymes. Because of radiations, enzymes are desactivated and cannot repair the DNA stucture of cells anymore. Abnormal cells can then prolifer, leading to different cancer forms. C-phycocyanin and polysaccharides contained in Spirulina may contribute to decrease radioactivity levels in ionised organisms.

Clinical studies

In the following study, spirulina (5g per day) was given to children affected by Tchernobyl radiations. The results showed that Spirulina reduced urine radioactivity levels by 50% in only 20 days.

L.P. Loseva and I.V. Dardynskaya.Spirulina- natural sorbent of radionucleides. Research Institute of Radiation Medicine, Minsk, Belarus. 6th Int'l Congress of Applied Algology, Czech Republic. Belarus. Sep 1993

In children living in highly radioactive areas, immunoglobulin E (IgE) was greatly above normal. Studies with 270 children showed that the consumption of 5 grams per day of spirulina normalized IgE within 6 weeks. IgE level remained unchanged in children who dit not consume spirulina. No side effects were observed.

Evets L., et al. Means to normalize the levels of immunoglobulin E, using the food supplement Spirulina. Grodenski State Medical Univ. Russian Federation Committee of Patents and Trade. Patent (19) RU (11)2005486. Jan. 15, 1994.

In a study on normal, gamma- irradiated and anemic mice, C-phycocyanin and polysaccharide of Spirulina were found to stimulate recovery of white blood cells and bone marrow cell counts. The anemic condition induced by irradiation was also reduced.

Zhang Cheng-Wu, Tseng Chao-Tsi & Zhang Yuan Zhen. The effect of polysaccharide and phycocyanin from Spirulina platensis var. on peripheral blood and hematopoietic system of bone marrow in mice. Paper presented at the 2nd Asia-Pacific Conference on Algal Biotechnology. Malaysia1994.

5. Conclusion

Traditional therapies always rely on the use of natural products and have been the source of information for the discovery of many drugs. Due to its rich contents of essential and non essential amino acids, polyunsaturated fatty acids (gamma-linolenic acid), vitamins as well as minerals, pigments and enzymes, Spirulina has numerous demonstrated pharmacological activities.

Mechanisms of anticancer, antiviral and antimicrobial effects of Spirulina are due to its content of endonuclease (which repair damaged DNA), calcium sulfated polysaccharide (which inhibits in vitro replication of viruses) and fatty acids (especially high content of gamma-linolenic acid), respectively. In addition, the metalloprotective role of Spirulina may be attributed to the presence of beta-carotene, vitamins C and E, enzyme superoxide dismutase, selenium and phycocyanin. Research has also focused on the immunostimulant effects of Spirulina. Some experimental observations indicate that phycocyanin, sulfated polysaccharide fractions, GLA and certain sulfolipids are the most promising active constituents of Spirulina.

In conclusion, numerous studies on Spirulina have been conducted in vitro, in vivo, on animals and humans. All these studies indicate the health benefits of Spirulina and stress the evidence for its therapeutic application in the following areas:

- Immune properties
- Antiallergic properties
- Antioxidant properties
- Anticancer properties
- Anti-viral properties
- Antibacterial properties
- Digestive properties
- Anti-diabetes properties (blood sugar level and insulin resistance lowering properties)
- Cholesterol lowering properties
- Antihypertensive) (high blood pressure lowering) properties
- Hepatoprotective properties
- Protection against atherosclerosis
- Protection against heavy metals
- Protection against radiation