REVIEW

Cardiovascular Therapeutics

Hypolipidemic, Antioxidant, and Antiinflammatory Activities of Microalgae *Spirulina*

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SUMMARY

Spirulina is free-floating filamentous microalgae growing in alkaline water bodies. With its high nutritional value, *Spirulina* has been consumed as food for centuries in Central Africa. It is now widely used as nutraceutical food supplement worldwide. Recently, great attention and extensive studies have been devoted to evaluate its therapeutic benefits on an array of diseased conditions including hypercholesterolemia, hyperglycerolemia, cardiovascular diseases, inflammatory diseases, cancer, and viral infections. The cardiovascular benefits of *Spirulina* are primarily resulted from its hypolipidemic, antioxidant, and antiinflammatory activities. Data from preclinical studies with various animal models consistently demonstrate the hypolipidemic activity of *Spirulina*. Although differences in study design, sample size, and patient conditions resulting in minor inconsistency in response to *Spirulina* supplementation, the findings from human clinical trials are largely consistent with the hypolipidemic effects of *Spirulina* observed in the preclinical studies. However, most of the human clinical trials are suffered with limited sample size and some with poor experimental design. The antioxidant and/or antiinflammatory activities of *Spirulina* were demonstrated in a large number of preclinical studies. However, a limited number of clinical trials have been carried out so far to confirm such activities in human. Currently, our understanding on the underlying mechanisms for *Spirulina*'s activities, especially the hypolipidemic effect, is limited. *Spirulina* is generally considered safe for human consumption supported by its long history of use as food source and its favorable safety profile in animal studies. However, rare cases of side-effects in human have been reported. Quality control in the growth and process of *Spirulina* to avoid contamination is mandatory to guarantee the safety of *Spirulina* products.

Introduction

Spirulina is referred to free-floating filamentous microalgae with spiral characteristics of its filaments. It is formally called *Arthrospira,* belonging to the class of cyanobacteria with characteristic photosynthetic capability [1,2]. *Spirulina* was initially classified in the plant kingdom because of its richness in plant pigments as well as its ability of photosynthesis. It was later placed in the bacteria kingdom based on new understanding on its genetics, physiology, and biochemical properties [3]. *Spirulina* naturally grows in high-salt alkaline water reservoirs in subtropical and tropical areas including America, Mexico, Asian, and Central Africa [3,4]. Among large number of *Spirulina* species, three species of *Spirulina*, including *Spirulina platensis* (*Arthrospira platensis*), *Spirulina maxima* (*Arthrospira maxima*), and *Spirulina fusiformis* (*Arthrospira fusiformis*) are most intensively investigated as those *Spirulina* species are edible with high nutritional as well as potential therapeutic values [3–6].

Early studies were mainly focused on the nutritional value of *Spirulina* as a food source. As early as over 400 years ago, *Spirulina* was eaten as food by the Mayas, Toltecs, and Kanembu in Mexico during the Aztec civilization [7]. *Spirulina* growing in the Lake Texcoco were harvested, dried and used to make *Spirulina* cake as food. It has also been over centuries for the Chadian to consume *Spirulina* in Central Africa. *Spirulina* harvested from the Lake Kossorom (Chat) is used to make cake or broths as meals and also sold on the market [8]. The nutritional value of *Spirulina* is well recognized with its unusual high protein content (60–70% by dry weight) and its richness in vitamins, minerals, essential fatty acids, and other nutrients [3,4]. Because of its unusual high nutritional values, the Intergovernmental Institution for the use of Micro-algae *Spirulina* Against Malnutrition (IIMSAM) was launched in the middle 1970s to promote *Spirulina* as high nutritional food to fight against starvation and malnutrition in the world [9]. In addition, due to its concentrated nutrition, *Spirulina* was recommended by both *National Aeronautics and Space*

Administration (NASA) and the European Space Agency (ESA) as one of the primary foods during long-term space missions.

Starting at middle 1980s, great efforts and extensive investigations have been turned to the development of nutraceuticals or functional food for preventing or managing various diseases. *Spirulina* has become one of such nutraceutical food with diverse beneficial effects on an array of disease conditions. It has been reported that consumption of *Spirulina* as diet supplement has health benefits in preventing or managing hypercholesterolemia, hyperglycerolemia, certain inflammatory diseases, allergies, cancer, environmental toxicant- and drug-induced toxicities, viral infections, cardiovascular diseases, diabetes, and other metabolic disease among others [5,6,10]. In this review, emphasis is given to the potential beneficial effects of *Spirulina* on cardiovascular diseases with highlights on *Spirulina*'s hypolipidemic, antioxidant, and antiinflamatory activities in preclinical and clinical studies. In addition, our current understanding on the mechanisms of action and the potential side-effects of *Spirulina* consumption are summarized.

Hypolipidemic Effects

Cholesterol is the building block for cell membrane and a precursor of steroid hormones. It forms several distinct particles with lipoproteins, mainly high-density lipoproteins (HDL), low-density lipoproteins (LDL), and very low-density lipoproteins (VLDL). It is well established that LDL and VLDL cholesterol levels are atherogenic whereas HDL-cholesterol has protective effects on the development of atherosclerosis [11,12]. Increased LDL and VLDL levels are the major independent risk factor for cardiovascular events whereas low level of HDL and elevated triglycerides (TG) are also recognized as residual risk for cardiovascular diseases [13]. Agents with the ability to decrease LDL/VLDL or total cholesterol levels, increase HDL cholesterol or lower TG have beneficial effects on preventing cardiovascular diseases.

Preclinical Studies

The hypolipidemic effect of *Spirulina* or its extracts have been demonstrated in various animal models including mouse, rat, hamster, and rabbit. The cholesterol lowering activity of *Spirulina* was first reported in albino rats [14], followed by in mice [15]. In the mouse study, supplementation of 16% *Spirulina* in a high fat and cholesterol diet resulted in a significant reduction in total serum cholesterol, LDL, VLDL cholesterol, and phospholipids whereas serum HDL cholesterol was concurrently increased. In addition, high hepatic lipids induced by the high fat and cholesterol diet were markedly reduced by *Spirulina* consumption [15].

Since the initial report of hypolipidemic effects of *Spirulina*, several *in vivo* studies were carried out in rats and mice under various experimentally induced conditions. In one study [16], hyperlipidemia was induced in Wistar rats by a high fructose (68%) diet. Inclusion of increasing percentages of *Spirulina* (5%, 10%, and 15%) in the diet significantly improved the hyperlipidemic profiles. Correlating with such improvement in lipid profiles, *Spirulina* feeding resulted in a significant increase in lipoprotein lipase and hepatic triglyceride lipase activity. Such increased lipase activity by *Spirulina* was suggested as a mechanism for improving the hyperlipidemia induced by high fructose diet. In another study with rats [17], fatty liver was induced by intraperitoneal injection of carbon tetrachloride (CCl_4), resulting in an increase in liver total cholesterol and triacylglycerols. However, such increases were significantly reduced by feeding oil extracts of *Spirulina* or defatted fraction of *Spirulina*. In addition, CCl_4-induced increase in total cholesterol level was completely prevented by feeding a diet containing whole *Spirulina*. A similar study was performed in CD-1 mice [18]. Fatty liver was induced by a daily dose of simmvastatin (75 mg/kg body weight) for 5 days with a high cholesterol diet and 20% ethanol in the drinking water. Serum and hepatic triacylglycerols, total lipids and cholesterol were all significantly increased. However, *Spirulina* feeding for 2 weeks prior to the onset of fatty liver induction decreased hepatic total lipids by 40%, triacylglycerols by 50%, and serum triacylglycerols by 45%, accompanied by a 45% increase in serum HDL cholesterol. The hypolipidemic activity of *Spirulina* was also confirmed in a diabetic mouse model [19]. Diabetic condition was induced by administration of alloxan (250 mg/kg body weight), resulting in evident fatty liver accompanied by altered serum and hepatic triacylglycerols and cholesterol levels. However, mice receiving a diet containing 5% *Spirulina* 1 week after the administration of alloxan for 4 weeks totally prevented fatty liver production, decreased serum and hepatic triacylglycerols, and fully or partially normalized HDL, LDL, and VLDL cholesterol levels. The study also showed that female mice were more resistant to diabetes induction by alloxan whereas more responsive to *Spirulina* treatment than male mice.

The hypolipidemic effects of *Spirulina* observed in mice and rats were verified in two recent studies with hamsters [20] and rabbits [21]. A group of hamsters fed an atherogenic diet supplemented with *Spirulina* or its ingredient phycocyanin exhibited lower total cholesterol, LDL, and VLDL cholesterol whereas HDL cholesterol was not affected. Furthermore, aortic fatty streak area was significantly reduced in hamsters receiving *Spirulina* supplement, indicating the antiatherogenic activity of *Spirulina* [20]. In the study with rabbits, hypercholesterolemia was induced by a high cholesterol diet and the effects of feeding *Spirulina* (0.5 g daily) for 30 and 60 days on the induced hypercholesterolemia was evaluated [21]. At the end of the study, serum total cholesterol was decreased by 49% while HDL cholesterol was increased by 25%. No significant changes in serum triacylglycerols were observed.

Taken together, the results from studies with various animal models consistently demonstrate the hypolipidemic activity of *Spirulina*, lowering serum total cholesterol, LDL, and VLDL fractions. In addition, other improvements in lipid profile were also observed in certain studies, including an increase in HDL cholesterol levels, decrease in atherogenic indices and triacylglycerol levels.

Clinical Studies

A number of human clinical trials have been performed to evaluate the hypolipidemic activity of *Spirulina* (Table 1). The target populations include healthy volunteers, patients with ischaemic heart disease, type 2 diabetes and nephrotic syndrome, and elderly subjects with or without hypercholesterolemic condition.

Table 1 Hypolipidemic effects of *Spirulina* in human clinical studies

Subject	Sample Size	Dose of *Spirulina*	Duration	Effects of *Spirulina*	Reference
Healthy volunteers (Male)	30	4.2 g, Daily	4 or 8 weeks	Total serum cholesterol and LDL were reduced significantly. Triglyceride levels decreased slightly whereas HDL-cholesterol showed no significant changes. The reduction of serum cholesterol was even greater in those men with the highest cholesterol levels.	[22]
Patinets with ischaemic heart disease	30	2 or 4 g, Daily	3 months	Total plasma cholesterol, LDL, VLDL and triglycerides were significantly reduced by 22.4% (2 g group)/33.5% (4 g group), 31%/45%, 22%/23%, and 22%/23%, respectively. HDL was significantly increased by 11.5%/12.8%. In addition, a significant reduction in body weight was achieved in both treatment groups.	[24]
Patients with type 2 diabetes mellitus	15	2 g, Daily	2 months	A significant reduction was detected in triglycerides, total cholesterol and free fatty acid levels. LDL and VLDL were also decreased. In addition, blood sugar and glycated serum protein levels were significantly decreased.	[25]
Patients with type 2 diabetes mellitus	25	2 g, Daily	2 months	Total serum cholesterol and LDL fraction were reduced whereas HDL was slightly increased. As a result, a significant decrease in atherogenic indices and the ratios of total cholesterol/HDL and LDL/HDL was observed. Furthermore, triglycerides and fasting and postprandial blood glucose levels were significantly reduced. Finally, the level of apolipoprotein B showed a significant fall with a concurrent significant increase in the level of apolipoprotein A1.	[26]
Patients with nephrotic syndrome	23	1 g, Daily	2 months	Total serum cholesterol, LDL cholesterol and triglycerides were all significantly decreased by 46 mg/dL, 33 mg/dL, and 45 mg/dL, respectively. The ratios of LDL/HDL and total cholesterol/HDL were also decreased significantly.	[29]
Healthy volunteers	36	4.5 g, Daily	6 weeks	Total plasma cholesterol and triacylglycerols were significantly reduced by 10% and 28%, respectively. HDL was significantly increased by 15% whereas LDL cholesterol was significantly decreased. In addition, both systolic and diastolic blood pressures were significantly reduced in both men and women.	[23]
Healthy elderly volunteers	12	7.5 g, Daily	24 weeks	The plasma concentrations of triglycerides, total cholesterol and LDL cholesterol were decreased after 4 weeks of the supplementation. No differences in hypolipidemic effects of *Spirulina* were observed between mild hypercholesterolemic (cholesterol at or above 200 mg/dL and normocholesterolemic subjects.	[33]
Elderly Women with hyperchole-sterolemia	51	7.5 mg, Daily	8 weeks	Serum levels of total cholesterol, LDL cholesterol and oxidized LDL were significantly reduced. In addition, apolipoprotein B, IL-6, and IL-6 production by peripheral blood lymphocyte were also decreased.	[34]
Patients with type 2 diabetes mellitus	37	8 g, Daily	12 weeks	Total serum cholesterol, LDL fraction and triglycerides were reduced with the subjects with higher initial total cholesterol, LDL-cholesterol and triglycerides showing higher reduction. In addition, blood pressure and IL-6 levels were also decreased. Finally, a significant reduction in plasma malondialdehyde level was observed.	[27]

Table 1 Continued

Subject	Sample Size	Dose of *Spirulina*	Duration	Effects of *Spirulina*	Reference
Patients with type 2 diabetes mellitus	60	1 or 2 g, Daily	2 months	A significant decrease was observed in serum total cholesterol, triglycerides, LDL, and VLDL cholesterol in *Spirulina* treatment groups. In addition, both fasting and postprandial blood glucose levels were also significantly decreased accompanied with decreased mean carbohydrate and protein intake.	[28]
Healthy elderly volunteers	78	8 g, Daily	16 weeks	Total plasma cholesterol and LDL fraction were significantly reduced in female subjects whereas a significant lowering effect on plasma total cholesterol by repeated test for treatment was observed in male subjects. However, no significant effect was detected in LDL fraction in male subjects. The levels of HDL fraction and triglycerides did not change after the intervention in both men and women	[35]

The first human study was carried out in 1988 with 30 healthy male volunteers with mild hyperlipidemia or hypertention [22]. The 30 subjects were divided into two groups; one group received 4.2 g of *Spirulina* daily for 8 weeks whereas the other group was given *Spirulina* for 4 weeks, followed by on regular food for another 4 weeks. Intake of *Spirulina* for 4 or 8 weeks significantly decreased total serum cholesterol and the decrease was more marked in mild hypercholesterolemic than in normocholesterolemic subjects. Discontinuation of *Spirulina* supplement for 4 weeks resulted in returning of the cholesterol level to the baseline (prior to *Spirulina* supplementation) and HDL levels were slightly increased but not statistically significant. There were no changes in serum triglycerides and body weight. In addition, no subjects reported adverse effects during the study. In a recent before-and-after clinical trial with 36 healthy volunteers (16 male and 20 female) between ages 18 to 65 [23], ingestion of *Spirulina* at a dose of 4.5 g daily for 6 weeks decreased total plasma cholesterol and triacylglycerols by 10% and 28%, respectively. Lipoprotein analysis showed that HDL cholesterol was increased by 15% whereas LDL cholesterol was significantly decreased. In addition, both systolic and diastolic blood pressures were significantly reduced in both men and women.

The hypolipidemic effect of *Spirulina* was also demonstrated in ischaemic heart disease patients with hypercholesterolemic condition (serum total cholesterol levels above 250 mg/dL) [24], a total of 30 patients were divided into three groups. Two treatment groups received 2 or 4 g of *Spirulina* daily for 3 months whereas control group was not supplemented with *Spirulina*. At the end of the supplementation, plasma total cholesterol was significantly decreased by 22.4% and 33.5% in groups receiving 2 and 4 g *Spirulina*, respectively, whereas no significant change was detected in the control group. Lipoprotein fraction analysis showed that LDL and VLDL cholesterol levels were significantly reduced by 31% and 45%, and 22% and 23% in the two treatment groups, respectively. On the other hand, HDL was significantly increased by 11.5% and 12.8%. Furthermore, the concentration of triglycerides was significantly reduced by 22% and 23%. Finally, a significant loss in body weight was observed in both treated groups whereas no change was detected in the control group. Thus, it was concluded that supplementation of *Spirulina* at a daily dose of 2 or 4 g for 3 months significantly improved the lipid profile of the patients with ischaemic heart disease.

Noninsulin-dependent diabetes mellitus (NIDDM) or type 2 diabetes mellitus is a recognized independent risk factor for cardiovascular diseases, such as coronary artery disease. The distinction between type 2 diabetes mellitus and cardiovascular disease has been blurred and prevention of cardiovascular diseases is becoming an integrated part of diabetes management. Patients with type 2 diabetes are frequently affected by atherosclerotic vascular disease. The abnormalities of both quantity and quality of lipoproteins in type 2 diabetes patients contribute to an increase in atherosclerotic vascular disease. So far, four human clinical studies have been performed to investigate the hypolipidemic and hyperglycerolemic effects of *Spirulina* in type 2 diabetic patients [25–28]. The two early studies were carried out by Dr. Iyer's group in India [25,26]. In a before-and-after study with 15 type 2 diabetes patients [25], supplementation of *Spirulina* at a dose of 2 g daily for 2 months resulted in a significant decrease in total serum cholesterol, triglycerides, and free fatty acid levels. Analysis of lipoprotein fractions revealed that LDL and VLDL cholesterol levels were appreciably reduced. Blood sugar and glycated serum protein levels were also significantly decreased. In a second randomized and controlled study [26], 25 patients with type 2 diabetes mellitus were randomly assigned to a study or control group. Subjects in the study group received *Spirulina* at a dose of 2 g/day for 2 months. At the end of the study, total serum cholesterol and LDL fraction were reduced whereas HDL was slightly increased in the study group. As a result, a significant decrease in atherogenic indices and the ratios of total cholesterol/HDL and LDL/HDL was achieved. Triglycerides and fasting and postprandial blood glucose levels were significantly reduced. Finally, the level of apolipoprotein B showed a significant fall with a concurrent significant increase in the level of apolipoprotein A1. Thus, the hypolipidemic and hypoglycerolemic effects of *Spirulina* were consistently detected in both clinical studies with type 2 diabetic patients.

The findings from the early studies were confirmed in the two recent human clinical trials with type 2 diabetic patients [27,28]. Both trials were randomized, controlled studies with a relatively

large sample size. One study enrolled 37 patients being randomly divided into a treatment or control group [27]. Intake of *Spirulina* at a dose of 8 g daily for 12 weeks significantly reduced total serum cholesterol, LDL fraction, and triglyceride levels. Subjects with higher initial total cholesterol, LDL-cholesterol, and triglyceride levels showed higher reduction. In addition, blood pressures were also decreased. The second trial included 60 male patients aging from 40 to 60 years [28]. The subjects were randomly assigned into two treatment groups or a control group. The two treatment groups received 1 or 2 g *Spirulina* daily for 2 months. A significant decrease was observed in serum total cholesterol, triglycerides, LDL and VLDL cholesterol in the two treatment groups. Both fasting and postprandial blood glucose levels were also decreased by 16.3% and 12.5% in 1 g-treated group and by 21.8% and 18.9% in 2 g-treated group whereas no significant changes were detected in the control group. It was also found that mean carbohydrate and protein intake was significantly decreased in both treatment groups. Taken together, the data are consistent with the notion that *Spirulina* is a promising agent as a functional food supplement for controlling hyperglycerolemia and hypercholesterolemia and thus reducing cardiovascular risk in the management of type 2 diabetes.

The hypolipidemic benefit of *Spirulina* was also reported in patients with nephrotic syndrome and hyperlipidemia [29]. One group of patients received medication alone whereas the other group received medication and *Spirulina* capsules. Supplementation of *Spirulina* at a dose of 1 g daily for 2 months resulted in a reduction in total serum cholesterol, LDL fraction and triglycerides by 46, 33, and 45 mg/dL, respectively. The ratios of LDL/HDL and total cholesterol/HDL were also decreased significantly. It was thus concluded that *Spirulina* supplementation was an effective approach to reduce the increased levels of lipids in patients with hyperlipidemic nephrotic syndrome.

Total and LDL cholesterol levels increase with aging [30,31] as does the incidence of cardiovascular disease [32]. Three human clinical studies have been carried out to investigate the therapeutic effects of *Spirulina* in elderly population [33–35]. In one study with 12 subjects (6 male and 6 female) between the ages 60 and 75 [33], subjects received a supplement of *Spirulina* at a dose of 7.5 g/day for 24 weeks. Plasma concentrations of triglycerides, total cholesterol and LDL fraction were decreased after 4 weeks of the supplementation while no changes were observed in dietary intake and anthropometric parameters. It was also noticed that no differences in the hypolipidemic effects of *Spirulina* were observed between mild hypercholesterolemic (cholesterol at or above 200 mg/dL) and normocholesterolemic subjects (cholesterol below 200 mg/dL). The second before-and-after trial included 26 elderly women aged over 60 with hypercholesterolaemic condition (serum total cholesterol above 200 mg/dL) [34]. Intake of *Spirulina* at a dose of 7.5 mg/day for 8 weeks resulted in a significant reduction in serum levels of total cholesterol, LDL cholesterol and oxidized LDL. In addition, apolipoprotein B levels were also decreased. The most recent clinical trial was a randomized, double-blinded, and placebo-controlled study [35]. Seventy-eight subjects between the ages 60 and 87 were randomly assigned into a study or placebo group. After consumption of *Spirulina* at a dose of 8 g/day for 16 weeks, total plasma cholesterol and LDL fraction were significantly reduced in female subjects whereas the lowering effect on plasma total cholesterol and LDL fraction was not statistically significant in male subjects. The levels of HDL fraction and triglycerides did not change after the intervention in both men and women. The data from those clinical trials largely support the notion that *Spirulina* supplement is beneficial for managing aging-induced alterations in lipid profile in the elderly population.

Taken together, although differences in study design, sample size and patient conditions resulting in minor inconsistency in response to *Spirulina* supplementation, the cumulative data from those studies clearly demonstrate the hypolipidemic activity of *Spirulina* in human. However, the majority of those human clinical trials are suffered with limited sample size and poor experimental design. Additional clinical trials with large sample size and high quality experimental design are warranted to confirm the hypolipidemic and hypoglycerolemic benefits of *Spirulina* in various target populations.

Antioxidant and Antiinflammatory Effects

Oxidative stress and inflammation both contribute to the pathogenesis of cardiovascular diseases, including atherosclerosis, cardiac hypertrophy, heart failure and hypertension. Overproduction of reactive oxygen species (ROS) indicating the oxidative stress have been observed in those cardiovascular disease conditions [36]. ROS also contributes to vascular dysfunction and remodeling through oxidative damages in endothelial cells [37]. In addition, evidence indicates that LDL oxidation is essential for atherogenesis [38,39]. On the other hand, the microenvironment present within the atherosclerotic lesion is proinflammatory. In addition to being a disorder of lipid metabolism, atherosclerosis is now recognized as a chronic inflammatory disease [40,41]. Accumulating evidence demonstrates that excessive inflammation within the arterial wall is a risk factor for cardiovascular diseases and can promote atherogenesis. Agents with antioxidant and/or antiinflammatory activity may prove to be beneficial in combating cardiovascular diseases.

Preclinical Studies

In Vitro Studies

A number of studies have reported the antioxidant and/or antiinflammatory activities of *Spirulina* or its extracts *in vitro* and *in vivo*, suggesting that *Spirulina* may provide a beneficial effect in managing cardiovascular conditions. In a study with neuroblastoma SH-SY5Y cells [42], the effects of *Spirulina* protean extract on iron-induced oxidative stress were investigated. *Spirulina* treatment protected the activity of the cellular antioxidant enzymes including glutathione peroxidase (GPX), selenium-dependent glutathione peroxidase (GPX-Se), and oxidized glutathione reductase (GR), and increased glutathione levels reduced in response to iron insult. The results clearly demonstrated the antioxidant activity of *Spirulina* extract. In a recent *in vitro* study [43], the antioxidant and antiinflammatory properties of four different *Spirulina* preparations were evaluated with a cell-free as well as a cell-based assay. It was found that *Spirulina* dose-dependently inactivated free

superoxide radicals generated during an oxidative burst. Equally significant, *Spirulina* dose-dependently reduced the metabolic activity of functional neutrophils, indicating the antiinflammatory activity.

Tissue homogenates were used in several *in vitro* studies to assess the antioxidant activity of *Spirulina*. In an early study [44], the antioxidant effect of methanolic extract of *Spirulina* on spontaneous lipid peroxidation of rat brain homogenate was investigated. It was showed that *Spirulina* extract dramatically inhibited the production of thiobarbituric acid reactive substances (TBARS), such as malondialdehyde (MDA), by almost 95%, indicating the potent antioxidant activity of *Spirulina*. Fluorouracil (5-FU) is an anticancer drug with cardiac toxicity and such cardiotoxicity is resulted from 5-FU-induced impairment in the myocardial antioxidant defense system, leading to cardiac peroxidation [45]. To evaluate the protective effects of *Spirulina* on 5-FU-induced lipid peroxidation, liver homogenate from goat was exposed to 5-FU or 5-FU and *Spirulina* water extract [46]. As expected, 5-FU caused an increase in biomarkers of lipid peroxidation, MDA and 4-hydroxy-2-nonenal (4-HNE), and a decrease in glutathione and nitric oxide content. However, *Spirulina* water extract significantly reduced the levels of MDA and 4-HNE, and increased the reduced content of glutathione. It was thus concluded that water extract of *Spirulina* significantly suppressed 5-FU-induced lipid peroxidation.

Cardiovascular diseases, such as hypertention, atherosclosis, and ischemic injury, are associated with altered endothelium function [47]. Vascular tone modification is an important function of the endothelium achieved by the synthesis and release of either vasodilating or vasoconstricting agents. Studies have demonstrated a dysfunction in nitric oxide synthesis and release, and an increased secretion of endothelium derived contracting factors in those disease conditions [48,49]. On the other hand, both LDL and oxidized-LDL are inhibitors of the endothelium dependent vasodilator responses [50]. Two studies with rat aorta rings were carried out to evaluate the effects of *Spirulina* on vascular tone. In one study [51], ethanol extract of *Spirulina* dose-dependently decreased the contractile response of the aortic ring to vasoconstricting agent phenylephrine (PE) whereas enhanced the relaxation response to vasodilating agent carbachol, consistent with the notion that *Spirulina* extract increased the basal synthesis and/or release of nitric oxide by the endothelium and cyclooxygenase-dependent vasoconstricting prostanoid by vascular smooth muscle cells. Similar findings were obtained in a recent study with aortic rings from fructose-induced obese rats [52]. Ethanolic extract of *Spirulina* significantly decreased PE-induced vasocontriction in a dose-dependent manner whereas no effects on carbachol-induced vasodilation were observed. The results suggested that ethanolic *Spirulina* extract increased the synthesis and release of nitric oxide but inhibited the synthesis and release of a cyclooxygenase-dependent vasoconstrictor metabolite of arachidonic acid.

In Vivo Studies

A number of animal studies have been carried out to evaluate the antioxidant and/or antiinflammatory activities of *Spirulina*. In one study with aged male rats [53], *Spirulina* reversed age-related increase in proinflammatory cytokines in cerebellum, such as tumor necrosis factor-alpha (TNFα) and TNFβ. *Spirulina* supplementation also significantly decreased the oxidative marker MDA whereas increased the cerebellar beta-adrenergic receptor function which was reduced by aging. The data thus demonstrated the antioxidant and antiinflammatory activities of *Spirulina* in aged rats.

Doxorubicin (DOX) is an anthracyclin antibiotic primarily used in the treatment of cancers. However, its application is limited due to its cardiac toxicity. The generation of ROS, lipid peroxidation, iron-dependent oxidative damage leading to mitochondrial dysfunction have been implicated in DOX-induced cardiotoxicity [54,55]. To determine whether *Spirulina* has cardioprotective activity in DOX-induced cardiotoxicity, mice were treated with DOX alone or DOX with *Spirulina* [56]. As expected, mice administrated with DOX exhibited severe cardiac pathologies. However, feeding of *Spirulina* at a dose of 250 mg/kg significantly decreased the mortality, ascites and lipid peroxidation; normalized the antioxidant enzymes levels; and minimized the microscopic damages to the heart. The data indicated that *Spirulina* had a protective effect on cardiotoxicity induced by DOX, most likely through its antioxidant activity.

As described previously, *Spirulina* extracts increased the basal synthesis and release of nitric oxide and cyclooxygenase-dependent vasoconstricting agent prostanoid by the endothelium *in vitro* [51,52]. Such findings were confirmed in two animal studies *in vivo*. In one early study with rats [57], feeding of a controlled diet containing 5% *Spirulina* significantly decreased the maximal tension of the aorta rings developed in response to vasoconstrictor PE. On the other hand, supplementation with *Spirulina* significantly increased the maximal relaxation in response to vasodilating agent carbachol. The data thus indicated that *Spirulina* increased the synthesis and release of endogenous vasodilating agents, such as nitro oxide, whereas decreased the synthesis and release of vasoconstricting agents, such as eicosanoid, leading to decreased vascular tone. Consistent results were obtained from another study with rats [58], in which feeding a diet containing 5% *Spirulina* prevented the decrease of the endothelium-dependent vasodilator responses of the aorta rings induced by a high fructose intake.

In addition, a large number of animal studies were carried out to investigating the preventive or protective effects of *Spirulina* intake on environmental toxicant, chemical, heavy metal or drug-induced oxidative stress and inflammation. Those studies were summarized in Table 2 [59–73]. Accumulative data from those studies concluded that *Spirulina* ingestion significantly relieved or totally prevented the oxidative stress or inflammation, and their associated pathological damages induced by insulting compounds. Although those studies were not directly investigating *Spirulina*'s effects on cardiovascular conditions, the findings clearly demonstrated the antioxidant and antiinflammatory activities of *Spirulina*.

Clinical Studies

In contrast to numerous preclinical studies, a limited number of clinical trials have been carried to evaluate the antioxidant and/or antiinflammatory activities of *Spirulina* in human. In one

Table 2 Antioxidant and antiinflammatory activities of *Spirulina* in preclinical studies

Animal	Inducing agent	Dose of *Spirulina*	Duration	Effects of *Spirulina* or its extracts	References
Rats	Haloperi-dol	0.18 g/kg Daily	7 weeks	Enzymatic and nonenzymatic antioxidants were significantly improved, and the tardive dyskinesia induced by haloperidol was decreased	[59]
Rats	Cisplatin	0.5–1.5 g/kg Daily	5 days	Significantly and dose-dependently restored renal functions damaged by cisplatin. A decrease in lipid peroxidation with an increase in glutathione levels, superoxide dismutase, and catalase activities	[60]
Mice	Zymosan	0.1 or 0.4 g/kg Daily	8 days	The β-glucuronidase that had been increased by zymosan was significantly reduced. Histopathological and ultrastructural evaluation showed inhibition of the inflammatory reaction with no destruction of cartilage, well-preserved chondrocytes, normal rough endoplasmic reticulum and mitochondria	[61]
Mice	Cyclopho-sphamide and mitomycin-C	0.25, 0.5, or 1.0 g/kg Daily	5 days	All the three doses resulted in a significant reduction in chromosomal damage and lipid peroxidation with concomitant changes in antioxidants and detoxification systems.	[62]
Rats	Lead	1.5 g/kg Daily	30 days	A significant decrease in the levels of malondialdehyde, conjugated diene and hydroperoxide.	[63]
Rats	Collagen	0.4 g/kg Daily	Up to 45 days	Normalized the joint histopathology of collagen-induced arthritis and decreased lipid peroxidation. Serum albumin was significantly elevated accompanied with a decrease in the serum cholesterol, alkaline phosphatase and acid phosphatase activities	[64]
Rats	Cadmium	0.5 g/kg Daily	1 month	A marked decrease in lipid peroxidation accompanied with an increase in endogenous antioxidants levels. In addition, the cadmium-induced histopathological changes were minimized	[65]
Rats	Gentami-cin sulphate	1 g/kg Daily	7 days	Significant nephroprotection was achieved by decreasing lipid peroxidation and elevating the levels of glutathione, superoxide dismutase, GPX, NO. Histological examination confirmed the nephroprotective effects of *Spirulina*	[66]
Rats	CCl_4	5% of the diet	2 days	A significant reduction in carbon tetrachloride-induced elevation of serum aspartate aminotransferase and liver triacylglycerols values. In addition, a significant decrease in free fatty acids and thiobarbituric acid reactive substances was observed	[67]
Mice	Mercuric chloride	0.8 g/kg Daily	40 days	decreased LPO level, serum glutamate oxaloacetate and serum glutamate pyruvate transaminase activity along with increase in liver GSH level. The activities of antioxidants enzymes superoxide dismutase, catalase, and glutathione-S-transferase were also concomitantly restored to near normal level by *Spirulina* supplementation to mercuric chloride intoxicated mice.	[68]

Table 2 Continued

Animal	Inducing agent	Dose of *Spirulina*	Duration	Effects of *Spirulina* or its extracts	References
Mice	Complete freund's adjuvant	0.8 g/kg Daily	8 days	Adjuvant induced arthritis in the paw characterized by a significant increase in tissue marker lysosomal enzymes, glycoproteins and the paw volume. All those arthritic alterations were almost completely normalized by *Spirulina* ingestion	[69]
Mice	Cisplatin and urethane	0.25, 0.5, or 1 g kg Daily	5 days	Genotoxicity induced by cisplatin and urethane was protected by *Spirulina*, accompanied with a significant reduction in the extent of lipid peroxidation and a concomitant increase in the liver enzymatic (GPx, GST, SOD, CAT) and nonenzymatic (reduced glutathione) antioxidants	[70]
Rats	Compound 48/80 or antidinitroph-enyl (DNP) IgE	0.01–1.0 g/kg Daily	Single dose	*Spirulina* dose-dependently inhibited compound 48/80 or anti-DNP IgE induced allergic reactions. Serum histamine levels and histamine release from peritoneal mast cells (RPMC) were dose-dependently decreased by *Spirulina*. In addition, the level of cyclic AMP in RPMC was significantly increased by 70-fold and the anti-DNP IgE-induced tumor necrosis factor-α production was inhibited	[71]
Rats	Cyclospo-rine	0.5 g/kg Daily	17 days	*Spirulina* protected cyclosporine induced-nephrotoxicity, evidenced by a decrease in plasma urea and creatinine. A significant decrease in plasma and local tissue MDA was observed. In addition, histopathological analysis revealed that the severe isometric vacuolization and widening of the interstitium induced by cyclosporine were completely prevented by *Spirulina*.	[72]
Mice	1-methyl-4-phenyl-1,2,3,6-tetrahydr-opyridine (MPTP)	0.025, 0.05, 0.1, 0.15, or 0.2 g/kg Daily	22 days	At a dose of 0.15 g/kg, *Spirulina* partially reversed the dopamine-depleting effect of MPTP and completely blocked the oxidative stress induced by MPTP	[73]

study with 26 elderly women, intake of *Spirulina* 7.5 mg/day for 8 weeks significantly decreased serum IL-6 levels and IL-6 production from peripheral blood lymphocytes [34], demonstrating the antiinflammatory activity of *Spirulina*. In a recent randomized, double-blind and placebo-controlled study with 78 healthy elderly subjects [35], supplementation of *Spirulina* at a dose of 8 g/day for 16 weeks resulted in a significant rise in plasma interleukin (IL)-2 concentrations in both male and female subjects with a concurrent reduction in IL-6 concentration in male subjects, and an increase in superoxide dismutase activity in female subjects. Finally, a recent clinical trial with 37 type 2 diabetes patients revealed that *Spirulina* ingestion at a dose of 8 g daily for 12 weeks significantly reduced serum interleukin 6 (IL-6) and oxidative marker MAD levels [27]. The data from those studies demonstrated the antioxidant and antiinflammatory activities of *Spirulina in vivo*.

It is well established that exercise promotes the production of reactive oxygen and nitrogen species, which contribute to skeletal muscle fatigue and damage [74]. Two clinical trials were conducted to investigate the effects of *Spirulina* on preventing excise-induced skeletal muscle fatigue and damage through its antioxidant property. In one study with 16 student volunteers, intake of a diet containing 5% *Spirulina* for 3 weeks resulted in a significant reduction of plasma oxidative marker MDA with a concurrent increase in the blood superoxide dismutase activity [75]. In a recent study with nine male subjects [76], supplementation of *Spirulina* with a daily dose of 8 g for 4 weeks significantly prolonged the time to fatigue, reduced TBARS induced by excise, and increased the plasma glutathione, protein carbonyls, catalase, and total antioxidant capacity levels. In addition, ingestion of *Spirulina* also significantly decreased carbohydrate oxidation rate by 10.3% and increased fat oxidation rate by 10.9%. Taken together, the data indicated that supplementation of *Spirulina* had preventive effects on skeletal muscle fatigue and damage mainly through its antioxidant activity.

Allergic rhinitis is characterized by allergic airway inflammation and hyperresponsiveness to nonspecific stimuli, often involving

activation of mast cells by IgE. To investigate whether *Spirulina* has therapeutic effects on alleviating allergic rhinitis through its antiinflammatory and antioxidant activities, two human clinical studies were carried out with allergic rhinitis patients. In a randomized, double-blinded crossover study [77], intake of *Spirulina* at a dose of 2 g/day for 12 weeks reduced IL-4 levels by 32% released from phytohemagglutinin (PHA)-stimulated peripheral blood mononuclear cells whereas no significant changes were observed for interferon gamma (IFNγ) and IL-2. In another recent trial [78], *Spirulina* consumption significantly improved the allergic symptoms compared with placebo, including nasal discharge, sneezing, nasal congestion and itching. Thus it was concluded that *Spirulina* was clinically effective on managing allergic rhinitis through its antiinflammatory and/or antioxidant properties.

Mechanism of Action

Hypolipidemic Activity

Although the hypolipidemic effect of *Spirulina* has been demonstrated in preclinical and clinical studies, our understanding on its mechanism of action is almost totally lacking. The active ingredients in *Spirulina* responsible for the hypolipidemic activity remain to be identified. In a study with *S. platensis* concentrate (SPC), it was found that SPC could bind cholesterol metabolites bile acids and decreased cholesterol solubility. Feeding rats with SPC significantly increased fecal excretion of cholesterol and bile acid. It was thus proposed that decreases in intestinal cholesterol and bile acid absorption following SPC feeding may represent a mechanism for the hypocholesterolemic action of SPC [79].

Phycocyanin is a water soluble protein and enriched in *Spirulina*. Ingestion of phycocyanin preparation made from SPC resulted in a significant decrease in serum total cholesterol and atherogenic index whereas serum HDL cholesterol was concurrently increased. It was thus suggested that phycocyanin might be the active ingredient in *Spirulina* responsible for the hypolipidemic activity [79]. However, addition studies with highly purified or expressed phycocyanin are required to confirm the findings.

Antioxidant and Antiinflammatory Effects

Spirulina contains several active ingredients, notably phycocyanin and β-carotene that have potent antioxidant and antiinflammatory activities. The antioxidant and antiinflammatory properties of phycocyanin were first reported in 1998 [80,81] and confirmed by numerous studies thereafter [20,82–92]. Phycocyanin has the ability to scavenge free radicals, including alkoxyl, hydroxyl, and peroxyl radicals. It also decreases nitrite production, suppresses inducible nitric oxide synthase (iNOS) expression, and inhibits liver microsomal lipid peroxidation [20,80–92]. Using recombinant technology, phycocyanin protein has been expressed and the antioxidant activity is also demonstrated with the recombinant phycocyanin protein [93,94].

As antiinflammatory activities, phycocyanin inhibits proinflammatory cytokine formation, such as TNFα, suppresses cyclooxygeanase-2 (COX-2) expression and decreases prostaglandin E(2) production [83,85,88–90]. In addition, phycocyanin has been reported to suppress the activation of nuclear factor-κB (NF-κB) through preventing degradation of cytosolic IκB-α [89] and modulate the mitogen-activated protein kinase (MAPK) activation pathways, including the p38, c-Jun N-terminal kinase (JNK), and extracellular-signal-regulated kinase (ERK1/2) pathways [95,96].

Another ingredient of S*pirulina*, β-carotene, has been reported to have antioxidant and antiinflammatory activities [97–99]. In a study to compare β-carotene, vitamin E, and nitric oxide as membrane antioxidants, it was found that β-carotene protected against singlet oxygen-mediated lipid peroxidation [97]. Studies also showed that β-carotene inhibited the production of nitric oxide and prostaglandin E(2), and suppressed the expression of iNOS, COX-2, TNF-α, and IL-1β. Such suppression of inflammatory mediators by β-carotene is likely resulted from its inhibition of NF-κB activation through blocking nuclear translocation of NF-κB p65 subunit [98]. In addition, β-carotene suppressed the transcription of inflammatory cytokines including IL-1β, IL-6, and IL-12 in macrophage cell line stimulated by lipopolysaccharide (LPS) or IFNγ [99].

Safety Profile

Spirulina has been consumed as food by man for long time in Mexico and Central Africa and is currently used widely as nutraceutic food supplement, especially in Asian. Over the history of *Spirulina* use by human, it has been generally considered safe to ingest *Spirulina*. Consistent with such notion are the results from a number of animal studies. However, few clinical studies have been carried out to systemically establish the safety profile of *Spirulina* in human.

To determine whether *Spirulina* feeding have any side effects on the growth and development of embryo and fetus, four animal studies with pregnant rats have been conducted [100–103]. In one study [100], *Spirulina* was administrated to pregnant rats from days 1–14, 1–21, and 7–14 of gestation with increasing doses (from 0 to 30 g/100 g body weight). It was found that *Spirulina* feeding did not change the maternal and fetal weight. No teratogenicity was detected with consumption of *Spirulina* even with highest dose and longest duration. Consistent results were obtained with a similar study in pregnant rats [101]. Supplementation of *Spirulina* in the diet at the doses much higher than any anticipated human consumption did not cause any signs of embryotoxic effects. In another study with rats [102], the effects of *Spirulina* alone or in combinations with other supplements on pregnancy were investigated. Maximal maternal weight gain was associated with *Spirulina*/wheat gluten diet whereas wheat gluten diet resulted in least weight gain. Intake of diet containing *Spirulina* significantly increased litter size whereas birth weights of pups were comparable to those from other groups. Finally, a study with pregnant rats to assess the general reproductive performance showed that *Spirulina* feeding did not change body weight of male and female rats with no signs of toxicity and was not associated with any adverse effects on any measures of reproductive performance including fertility, gestation and abnormal pups [103]. Taken together, it was concluded that *Spirulina* had no detectable adverse effects

on reproductive performance, embryo and fetus development and growth [104].

To evaluate the safety of relatively long-term feeding of *Spirulina*, mice were fed with a diet containing increasing percentages (from 0% to 30% w/w) of *Spirulina* for 13 weeks [105]. Ingestion of *Spirulina* had no effects on behavior, food and water intake, growth, and survival. Hematologic and clinical chemistry analyses revealed no abnormality. In addition, no gross or microscopic changes were detected with histological evaluation. In a recent study with short and long-term *Spirulina* feeding in rodents [106], feeding mice with high dose of *Spirulina* (30 g and 10 g/kg body weight of fresh and dried *Spirulina*, respectively) for 7 days resulted in no signs of toxicity. In the long-term feeding study, rats were administrated with *Spirulina* at various doses for 12 weeks. Consumption of *Spirulina* did not cause any changes in behavior, food and water intake, growth, health status, and measurements of clinical chemistry [106].

Despite of favorable safety profile in rodents, there were reports raising the concerns of the safety of *Spirulina* consumption. A low level of mercury and other heavy metals were reported in *Spirulina* grown in open water source [107]. Consumption of such *Spirulina* preparation could lead to increased deposit of mercury and other heavy metals causing toxic effects. However, with controlled water sources for growing *Spirulina*, commercial *Spirulina* products tested contained mercury or lead at the levels much lower than the guidelines for daily intake of those elements by the WHO's Food and Agriculture Organization (FAO) [108]. Certain cyanobacterial species produce cyanotoxin and contamination of those species in the *Spirulina* products may be deleterious for consumers [109]. In a newly reported study, anatoxin-a, a cyanotoxin with acute neurotoxicity, were detected in 3 of the 39 cyanobacterial samples [110]. It was thus recommended that quality control of cyanobacterial food supplements including *Spirulina* was required to avoid potential adverse effects in animals and humans.

Finally, a few rare incidences associated with consumption of *Spirulina* supplements in human have been reported. One case of hepatotoxicity was possibly associated with *Spirulina* intake [111], although the patient also took three other medications. A case of rhabdomyolysis was recently reported as a result of *Spirulina* intake [112]. Finally, an association of *Spirulina* consumption and development of a mixed immunoblistering disorder with characteristic features of bullous pemphigoid and pemphigus foliaceus was reported in an 82-year-old healthy woman [113].

Taken together, *Spirulina* is generally considered safe for human consumption supported by its long history of use as food source, and its favorable safety profile in animal studies. However, rare cases of side-effects have been reported and should be taken into consideration. In addition, additional clinical studies are required to systemically establish the safety profile of *Spirulina* in human. Finally, quality control in the growth and process of *Spirulina* to avoid contamination is mandatory to guarantee the safety of *Spirulina* products.

Concluding Remarks

Recently, great attention and extensive studies have been devoted to evaluate the therapeutic benefits of *Spirulina* on various diseased conditions including hypercholesterolemia, hyperglycerolemia, cardiovascular diseases, inflammatory diseases, cancer, and viral infections. The cardiovascular benefits of *Spirulina* are primarily resulted from its hypolipidemic, antioxidant, and antiinflammatory activities. Data from preclinical studies with various animal models consistently demonstrate the hypolipidemic activity of *Spirulina*. Although differences in study design, sample size and patient conditions resulting in minor inconsistency in response to *Spirulina* supplementation, the findings from human clinical trials are largely consistent with the hypolipidemic effects of *Spirulina* observed in the preclinical studies. However, most of the human clinical trials are suffered with limited sample size and some with poor experimental design. Additional clinical trials with large sample size and high quality experimental design are warranted to confirm the hypolipidemic benefits of *Spirulina* in various target human populations.

The antioxidant and/or antiinflammatory activities of *Spirulina* have been demonstrated in a large number of preclinical studies. However, a limited number of clinical trials have been carried out so far to confirm such activities in human. Future clinical trials are required to establish the antioxidant and antiinflammatory benefits in human. In addition, efforts should be taken to standardize the dose of *Spirulina* in future human clinical studies. *Spirulina* is generally considered safe for human consumption supported by its long history of use as food source and its favorable safety profile in animal studies. However, rare cases of side-effects in human have been reported. Additional clinical studies are required to systemically establish the safety profile of *Spirulina* in human. Quality control in the growth and process of *Spirulina* is a key measurement to avoid contamination and guarantee the safety of *Spirulina* products. Currently, our understanding on the underlying mechanisms for *Spirulina*'s activities, especially the hypolipidemic effect, is still limited. Future studies to identify the active ingredients in *Spirulina* and uncover the mechanistic insights into *Spirulina*'s therapeutic effects will provide the bases for developing new drugs for preventing or treating hypercholesterolemia and cardiovascular diseases.

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Conflict of Interest

The authors declare no conflict of interests.

References

1. Sapp J. The prokaryote-eukaryote dichotomy: Meanings and mythology. *Microbiol Mol Biol Rev* 2005;**69**:292–305.
2. Komárek J, Hauer T. CyanoDB.cz—On-line database of cyanobacterial genera. *Worldwide electronic publication*, Univ. of South Bohemia and Inst of Botany AS CR 2009; http://www.cyanodb.cz.

3. Vonshak A. (editor). *Spirulina platensis (Arthrospira): Physiology, cell-biology and biotechnology*. London: Taylor & Francis, 1997.
4. Gershwin ME, Belay A (editors). *Spirulina in human nutrition and health*. Boca Raton: CRC Press, 2008.
5. Khan Z, Bhadouria P, Bisen PS. Nutritional and therapeutic potential of *Spirulina*. *Curr Pharm Biotechnol* 2005;**6**:373–379.
6. Karkos PD, Leong SC, Karkos CD, Sivaji N, Assimakopoulos DA. *Spirulina* in clinical practice: Evidence-based human applications. *Evid Based Complement Alternat Med* 2008;**eCAM**:1–4.
7. Ciferri O, Tiboni O. The biochemistry and industrial potential of *Spirulina*. *Ann Rev Microbiol* 1985;**39**:503–526.
8. Abdulqader G, Barsanti L, Tredici M. Harvest of Arthrospira platensis from Lake Kossorom (Chad) and its household usage among the Kanembu. *J Appl Phychol* 2000;**12**:493–498.
9. Habib MAB, Parvin M, Huntington TC, Hasan MR. A review on culture, production, and use of *Spirulina* as food for humans and feeds for domestic animals and fish. FAO Fisheries and Aquaculture *Circular* No.034, 2008.
10. Kulshreshtha A, Zacharia AJ, Jarouliya U, Bhadauriya P, Prasad GB, Bisen PS. *Spirulina* in health care management. *Curr Pharm Biotechnol* 2008;**9**:400–405.
11. National Cholesterol Education Program (NCEP). Expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III). Third report of the national cholesterol education program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III) final report. *Circulation* 2002;**106**:3143–3421.
12. Barter P, Gotto AM, LaRosa JC, et al.; Treating to New Targets Investigators. HDL cholesterol, very low levels of LDL cholesterol, and cardiovascular events. *N Engl J Med* 2007;**357**:1301–1310.
13. Sharma RK, Singh VN, Reddy HK. Thinking beyond low-density lipoprotein cholesterol: Strategies to further reduce cardiovascular risk. *Vasc Health Risk Manag* 2009;**5**:793–799.
14. Devi MA, Venkataraman LV. Hypocholesterolemic effect of blue-green algae *Spirulina platensis* in albino rats. *Ann Nutr Reports Int* 1983;**28**:519–530.
15. Kato T, Takemoto K, Katayama H, Kuwabara Y. Effects of *Spirulina (Spirulina platensis)* on dietary hypercholesterolemia in rats. *J Jap Soc Nutr Food Sci* 1984;**37**:323–332.
16. Iwata K, Inayama T, Kato T. Effects of *Spirulina platensis* on plasma lipoprotein lipase activity in fructose-induced hyperlipidemic rats. *J Nutr Sci Vitaminol (Tokyo)* 1990;**36**:165–171.
17. Torres-Durán PV, Miranda-Zamora R, Paredes-Carbajal MC, Mascher D, Blé-Castillo J, Díaz-Zagoya JC, Juárez-Oropeza MA. Studies on the preventive effect of *Spirulina maxima* on fatty liver development induced by carbon tetrachloride, in the rat. *J Ethnopharmacol* 1999;**64**:141–147.
18. Blé-Castillo JL, Rodríguez-Hernández A, Miranda-Zamora R, Juárez-Oropeza MA, Díaz-Zagoya JC. Arthrospira maxima prevents the acute fatty liver induced by the administration of simvastatin, ethanol and a hypercholesterolemic diet to mice. *Life Sci* 2002;**70**:2665–2673.
19. Rodríguez-Hernández A, Blé-Castillo JL, Juárez-Oropeza MA, Díaz-Zagoya JC. *Spirulina maxima* prevents fatty liver formation in CD-1 male and female mice with experimental diabetes. *Life Sci* 2001;**69**:1029–1037.
20. Riss J, Décordé K, Sutra T, et al. Phycobiliprotein C-phycocyanin from *Spirulina platensis* is powerfully responsible for reducing oxidative stress and NADPH oxidase expression induced by an atherogenic diet in hamsters. *J Agric Food Chem* 2007;**55**:7962–7967.
21. Colla LM, Muccillo-Baisch AL, Costa JAV. *Spirulina platensis* Effects on the Levels of Total Cholesterol, HDL and Triacylglycerols in Rabbits Fed with a Hypercholesterolemic Diet. *Braz Arch Biol Technol* 2008;**51**:405–411.
22. Nakaya N, Homa Y, Goto Y. Cholesterol lowering effect of *Spirulina*. *Nutr Rep Int* 1988;**37**:1329–1337.
23. Torres-Duran PV, Ferreira-Hermosillo A, Juarez-Oropeza MA. Antihyperlipemic and antihypertensive effects of *Spirulina maxima* in an open sample of mexican population: A preliminary report. *Lipids Health Dis* 2007;**6**:1–8.
24. Ramamoorthy A, Premakumari S. Effect of supplementation of *Spirulina* on hypercholesterolemic patients. *J Food Sci Technol* 1996;**33**:124–128.
25. Mani UV, Desai S, Iyer U. Studies on the long-term effect of *Spirulina* supplementation on serum lipid profile and glycated proteins in NIDDM patients. *J Nutraceut, Funct Med Foods* 2000;**2**:25–32.
26. 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;**4**:193–199.
27. Lee EH, Park JE, Choi YJ, Huh KB, Kim WY. A randomized study to establish the effects of *Spirulina* in type 2 diabetes mellitus patients. *Nutr Res Pract* 2008;**2**:295–300.
28. Kamalpreet K, Rajbir S, Kiran G. Effect of supplementation of *Spirulina* on blood glucose and lipid profile of the non-insulin dependent diabetic male subjects. *J Dairying, Foods Home Sci* 2008;**27**:3–4.
29. Samuels R, Mani UV, Iyer UM, Nayak US. Hypocholesterolemic effect of *Spirulina* in patients with hyperlipidemic nephrotic syndrome. *J Med Food* 2002;**5**:91–96.
30. Heiss G, Tamir I, Davis CE, Tyroler HA. Lipoprotein-cholesterol distributions in selected North American populations: The Lipid Research Clinics Program prevalence study. *Circulation* 1980;**61**:302–315.
31. Abbott RD, Garrison RJ, Wilson PW, Epstein FH, Castelli WP, Feinleib M, LaRue C. Joint distribution of lipoprotein cholesterol classes: The Framingham Study. *Arteriosclerosis* 1983;**3**:260–272.
32. Castelli WP, Wilson PW, Levy D, Anderson K. Cardiovascular risk factors in the elderly. *Am J Cardiol* 1989;**63**:12H–19H.
33. Park JY, Kim WY. The effect of *Spirulina* on lipid metabolism, antioxidant capacity and immune function in Korean elderly. *Korean J Nutr* 2003;**36**:287–297.
34. Kim MH, Kim WY. The change of lipid metabolism and immune function caused by antioxidant material in the hypercholesterolemin elderly women in Korea. *Korean J Nutr* 2005;**38**:67–75.
35. Park HJ, Lee YJ, Ryu HK, Kim MH, Chung HW, Kim WY. A randomized double-blind, placebo-controlled study to establish the effects of *Spirulina* in elderly Koreans. *Ann Nutr Metab* 2008;**52**:322–328.
36. Dhalla NS, Temsah RM, Netticadan T. Role of oxidative stress in cardiovascular diseases. *J Hypertens* 2000;**18**:655–673.
37. Yung LM, Leung FP, Yao X, Chen ZY, Huang Y. Reactive oxygen species in vascular wall. *Cardiovasc Hematol Disord Drug Targets* 2006;**6**:1–19.
38. Steinberg D. Low density lipoprotein oxidation and its pathobiological significance. *J Biol Chem* 1997;**272**:20963–20966.
39. Chisolm GM, Steinberg D. The oxidative modification hypothesis of atherogenesis: An overview. *Free Radic Biol Med* 2000;**28**:1815–1826.
40. Lusis AJ. Atherosclerosis. *Nature* 2000;**407**:233–241.
41. Glass CK, Witztum JL. Atherosclerosis. The road ahead. *Cell* 2001;**104**:503–516.
42. Bermejo-Bescós P, Piñero-Estrada E, Villar del Fresno AM. Neuroprotection by *Spirulina* platensis protean extract and phycocyanin against iron-induced toxicity in SH-SY5Y neuroblastoma cells. *Toxicol In Vitro* 2008;**22**:1496–1502.
43. Dartsch PC. Antioxidant potential of selected *Spirulina platensis* preparations. *Phytother Res* 2008;**22**:627–633.
44. Miranda MS, Cintra RG, Barros SB, Mancini Filho J. Antioxidant activity of the microalga *Spirulina* maxima. *Braz J Med Biol Res* 1998;**31**:1075–1079.
45. Simbre C, Duffy A, Dadlani H, Miller L, Lipshultz E. Cardiotoxicity of cancer chemotherapy: Implications for children. *Pediatr Drugs* 2005;**7**:187–202.
46. Ray S, Roy K, Sengupta C. In vitro evaluation of protective effects of ascorbic acid and water extract of *Spirulina plantesis* (blue green algae) on 5-fluorouracil-induced lipid peroxidation. *Acta Pol Pharm* 2007;**64**:335–344.
47. Landmesser U, Hornig B, Drexler H. Endothelial function: A critical determinant in atherosclerosis? *Circulation* 2004;**109**(Suppl 1):1127–1133.
48. Luscher TF. Endotheliumderived relaxing and contracting factors: Potential role in coronary artery disease. *Eur Heart J* 1989;**l**:847–857.
49. Miyauchi T, Yanagisawa M, Suzuki N, et al. Venous plasma concentrations of endothelin in normal and hypertensive subjects. *Circulation* 1989;**80**(Suppl II):2280.
50. Andrews HE, Bruckdorkr KR, Dunn RC, Jacobs M. Low density lipoproteins inhibit endotheliumdependent relaxation in rabbit aorta. *Nature* 1987;**327**:237–239.
51. Paredes-Carbajal MC, Torres-Durán PV, Díaz-Zagoya JC, Mascher D, Juárez-Oropeza MA. Effects of the ethanolic extract of *Spirulina maxima* on

endothelium dependent vasomotor responses of rat aortic rings. *J Ethnopharmacol* 2001;**75**:37–44.
52. Mascher D, Paredes-Carbajal MC, Torres-Durán PV, Zamora-González J, Díaz-Zagoya JC, Juárez-Oropeza MA. Ethanolic extract of *Spirulina maxima* alters the vasomotor reactivity of aortic rings from obese rats. *Arch Med Res* 2006;**37**:50–57.
53. Gemma C, Mesches MH, Sepesi B, Choo K, Holmes DB, Bickford PC. Diets enriched in foods with high antioxidant activity reverse age-induced decreases in cerebellar beta-adrenergic function and increases in proinflammatory cytokines. *J Neurosci* 2002;**22**:6114–6120.
54. Xu MF, Tang PL, Qian ZM, Ashraf M. Effects by doxorubicin on the myocardium are mediated by oxygen free radicals. *Life Sci* 2001;**68**:889–901.
55. Doroshow JH. Doxorubicin-induced cardiac toxicity. *N Engl J Med* 1991;**324**:843–845.
56. Khan M, Shobha JC, Mohan IK, et al. Protective effect of *Spirulina* against doxorubicin-induced cardiotoxicity. *Phytother Res* 2005;**19**:1030–1037.
57. Paredes-Carbajal MC, Torres-Durán PV, Díaz-Zagoya JC, Mascher D, Juárez-Oropeza MA. Effects of dietary *Spirulina maxima* on endothelium dependent vasomotor responses of rat aortic rings. *Life Sci* 1997;**61**:PL211–219.
58. Paredes-Carbajal MC, Torres-Durán PV, Rivas-Arancibia S, Zamora-Gonzalez J, Mascher D, Juárez-Oropeza MA. Effects of dietary *Spirulina maxima* on vasomotor responses of aorta rings from rats fed on a fructose-rich diet. *Nutr Res* 1998;**18**:1769–1782.
59. Thaakur SR, Jyothi B. Effect of *Spirulina maxima* on the haloperidol induced tardive dyskinesia and oxidative stress in rats. *J Neural Transm* 2007;**114**:1217–1225.
60. Kuhad A, Tirkey N, Pilkhwal S, Chopra K. Renoprotective effect of *Spirulina fusiformis* on cisplatin-induced oxidative stress and renal dysfunction in rats. *Ren Fail* 2006;**28**:247–254.
61. Remirez D, González R, Merino N, Rodriguez S, Ancheta O. Inhibitory effects of *Spirulina* in zymosan-induced arthritis in mice. *Mediators Inflamm* 2002;**11**:75–79.
62. Premkumar K, Pachiappan A, Abraham SK, Santhiya ST, Gopinath PM, Ramesh A. Effect of *Spirulina fusiformis* on cyclophosphamide and mitomycin-C induced genotoxicity and oxidative stress in mice. *Fitoterapia* 2001;**72**:906–911.
63. Upasani CD, Khera A, Balaraman R. Effect of lead with vitamin E, C, or *Spirulina* on malondialdehyde, conjugated dienes and hydroperoxides in rats. *Indian J Exp Biol* 2001;**39**:70–74.
64. Kumar N, Singh S, Patro N, Patro I. Evaluation of protective efficacy of *Spirulina platensis* against collagen-induced arthritis in rats. *Inflammopharmacol* 2009;**17**:181–190.
65. Karadeniz A, Cemek M, Simsek N. The effects of Panax ginseng and *Spirulina platensis* on hepatotoxicity induced by cadmium in rats. *Ecotoxicol Environ Saf* 2009;**72**:231–235.
66. Karadeniz A, Yildirim A, Simsek N, Kalkan Y, Celebi F. *Spirulina platensis* protects against gentamicin-induced nephrotoxicity in rats. *Phytother Res* 2008;**22**:1506–1510.
67. Torres-Dura'n PV, Paredes-Carbajal AMC, Mascher BD, Zamora-Gonza'lez BJ, Dı'az-Zagoya CJD, Jua'rez-Oropezaa AMA. Protective Effect of Arthrospira maxima on Fatty Acid Composition in Fatty Liver. *Arch Medl Res* 2006;**37**:479–483.
68. Sharma MK, Sharma A, Kumar A, Kumar M. *Spirulina fusiformis* provides protection against mercuric chloride induced oxidative stress in Swiss albino mice. *Food Chem Toxicol* 2007;**45**:2412–2419.
69. Rasool M, Sabina EP, Lavanya B. Anti-inflammatory effect of *Spirulina fusiformis* on adjuvant-induced arthritis in mice. *Biol Pharm Bull* 2006;**29**:2483–2487.
70. Premkumar K, Abraham SK, Santhiya ST, Ramesh A. Protective effect of *Spirulina fusiformis* on chemical-induced genotoxicity in mice. *Fitoterapia* 2004;**75**:24–31.
71. Kim HM, Lee EH, Cho HH, Moon YH. Inhibitory effect of mast cell-mediated immediate-type allergic reactions in rats by *Spirulina*. *Biochem Pharmacol* 1998;**55**:1071–1076.
72. Khan M, Shobha JC, Mohan IK, Rao Naidu MU, Prayag A, Kutala VK. *Spirulina* attenuates cyclosporine-induced nephrotoxicity in rats. *J Appl Toxicol* 2006;**26**:444–451.
73. Chamorro G, Pérez-Albiter M, Serrano-García N, Mares-Sámano JJ, Rojas P. *Spirulina maxima* pretreatment partially protects against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine neurotoxicity. *Nutr Neurosci* 2006;**9**:207–212.
74. Ferreira LF, Reid MB. Muscle-derived ROS and thiol regulation in muscle fatigue. *J Appl Physiol* 2008;**104**:853–860.
75. Lu HK, Hsieh CC, Hsu JJ, Yang YK, Chou HN. Preventive effects of *Spirulina platensis* on skeletal muscle damage under exercise-induced oxidative stress. *Eur J Appl Physiol* 2006;**98**:220–226.
76. Kalafati M, Jamurtas A, Nikolaidis MG, et al. Ergogenic and antioxidant effects of *Spirulina* supplementation in humans. *Med Sci Sports Exerc* 2010;**42**:142–151.
77. Mao TK, Van de Water J, Gershwin ME. Effects of a *Spirulina*-based dietary supplement on cytokine production from allergic rhinitis patients. *J Med Food* 2005;**8**:27–30.
78. Cingi C, Conk-Dalay M, Cakli H, Bal C. The effects of *Spirulina* on allergic rhinitis. *Eur Arch Otorhinolaryngol* 2008;**265**:1219–1223.
79. Nagaoka S, Shimizu K, Kaneko H, et al. A novel protein C-phycocyanin plays a crucial role in the hypocholesterolemic action of *Spirulina platensis* concentrate in rats. *J Nutr* 2005;**135**:2425–2430.
80. Romay C, Armesto J, Remirez D, González R, Ledon N, García I. Antioxidant and anti-inflammatory properties of C-phycocyanin from blue-green algae. *Inflamm Res* 1998;**47**:36–41.
81. Romay C, Ledón N, González R. Further studies on anti-inflammatory activity of phycocyanin in some animal models of inflammation. *Inflamm Res* 1998;**47**:334–338.
82. González R, Rodríguez S, Romay C, et al. Anti-inflammatory activity of phycocyanin extract in acetic acid-induced colitis in rats. *Pharmacol Res* 1999;**39**:55–59.
83. Romay C, Delgado R, Remirez D, González R, Rojas A. Effects of phycocyanin extract on tumor necrosis factor-alpha and nitrite levels in serum of mice treated with endotoxin. *Arzneimittelforschung* 2001;**51**:733–736.
84. Remirez D, Ledón N, González R. Role of histamine in the inhibitory effects of phycocyanin in experimental models of allergic inflammatory response. *Mediat Inflamm* 2002;**11**:81–85.
85. Remirez D, Fernández V, Tapia G, González R, Videla LA. Influence of C-phycocyanin on hepatocellular parameters related to liver oxidative stress and Kupffer cell functioning. *Inflamm Res* 2002;**51**:351–356.
86. Romay Ch, González R, Ledón N, Remirez D, Rimbau V. C-phycocyanin: A biliprotein with antioxidant, anti-inflammatory and neuroprotective effects. *Curr Protein Pept Sci* 2003;**4**:207–216.
87. Khan M, Varadharaj S, Shobha JC, Naidu MU, Parinandi NL, Kutala VK, Kuppusamy P. C-phycocyanin ameliorates doxorubicin-induced oxidative stress and apoptosis in adult rat cardiomyocytes. *J Cardiovasc Pharmacol* 2006;**47**:9–20.
88. Patel A, Mishra S, Ghosh PK. Antioxidant potential of C-phycocyanin isolated from cyanobacterial species Lyngbya, Phormidium and *Spirulina* spp. *Indian J Biochem Biophys* 2006;**43**:25–31.
89. Riss J, Décordé K, Sutra T, et al. Phycobiliprotein C-phycocyanin from *Spirulina platensis* is powerfully responsible for reducing oxidative stress and NADPH oxidase expression induced by an atherogenic diet in hamsters. *J Agric Food Chem* 2007;**55**:7962–7967.
90. Cherng SC, Cheng SN, Tarn A, Chou TC. Anti-inflammatory activity of c-phycocyanin in lipopolysaccharide-stimulated RAW 264.7 macrophages. *Life Sci* 2007;**81**:1431–1435.
91. Shih CM, Cheng SN, Wong CS, Kuo YL, Chou TC. Antiinflammatory and antihyperalgesic activity of C-phycocyanin. *Anesth Analg* 2009;**108**:1303–1310.
92. Manconia M, Pendás J, Ledón N, Moreira T, Sinico C, Saso L, Fadda AM. Phycocyanin liposomes for topical anti-inflammatory activity: In-vitro in-vivo studies. *J Pharm Pharmacol* 2009;**61**:423–430.
93. Ge B, Qin S, Han L, Lin F, Ren Y. Antioxidant properties of recombinant allophycocyanin expressed in Escherichia coli. *J Photochem Photobiol B* 2006;**84**:175–180.

94. Guan XY, Zhang WJ, Zhang XW, et al. A potent anti-oxidant property: Fluorescent recombinant alpha-phycocyanin of *Spirulina*. *J Appl Microbiol* 2009;**106**:1093–1100.
95. Khan M, Varadharaj S, Ganesan LP, et al. C-phycocyanin protects against ischemia-reperfusion injury of heart through involvement of p38 MAPK and ERK signaling. *Am J Physiol Heart Circ Physiol* 2006;**290**:H2136–H2145.
96. Li XL, Xu G, Chen T, et al. Phycocyanin protects INS-1E pancreatic beta cells against human islet amyloid polypeptide-induced apoptosis through attenuating oxidative stress and modulating JNK and p38 mitogen-activated protein kinase pathways. *Int J Biochem Cell Biol* 2009;**41**:1526–1535.
97. Schafer FQ, Wang HP, Kelley EE, Cueno KL, Martin SM, Buettner GR. Comparing beta-carotene, vitamin E and nitric oxide as membrane antioxidants. *Biol Chem* 2002;**383**:671–681.
98. Bai SK, Lee SJ, Na HJ, et al. beta-Carotene inhibits inflammatory gene expression in lipopolysaccharide-stimulated macrophages by suppressing redox-based NF-kappaB activation. *Exp Mol Med* 2005;**37**:323–334.
99. Katsuura S, Imamura T, Bando N, Yamanishi R. beta-Carotene and beta-cryptoxanthin but not lutein evoke redox and immune changes in RAW264 murine macrophages. *Mol Nutr Food Res* 2009;**53**:1396–1405.
100. Chamorro G, Salazar M, Salazar S. Teratogenic study of *Spirulina* in rats. *Arch Latinoam Nutr* 1989;**39**:641–649.
101. Chamorro G, Salazar M. Teratogenic study of *Spirulina* in mice. *Arch Latinoam Nutr* 1990;**40**:86–94.
102. Kapoor R, Mehta U. Effect of supplementation of blue green alga (*Spirulina*) on outcome of pregnancy in rats. *Plant Foods Hum Nutr* 1993;**43**:29–35.
103. Salazar M, Chamorro GA, Salazar S, Steele CE. Effect of *Spirulina maxima* consumption on reproduction and peri- and postnatal development in rats. *Food Chem Toxicol* 1996;**34**:353–359.
104. Chamorro G, Salazar M, Favila L, Bourges H. Pharmacology and toxicology of *Spirulina* alga. *Rev Invest Clin* 1996;**48**:389–399.
105. Salazar M, Martínez E, Madrigal E, Ruiz LE, Chamorro GA. Subchronic toxicity study in mice fed *Spirulina maxima*. *J Ethnopharmacol* 1998;**62**:235–241.
106. Hutadilok-Towatana N, Reanmongkol W, Satitit S, Panichayupakaranant P, Ritthisunthorn P. A subchronic toxicity study of *Spirulina platensis*. *Food Sci Technol Res* 2008;**14**:351.
107. Johnson PE, Shubert LE. Accumulation of mercury and other elements by *Spirulina* (cyanophyceae). *Nutr Rep Int* 1986;**34**:1063–1070.
108. Slotton DG, Goldman CR, Franke A. Commercially grown *Spirulina* found to contain low levels of mercury and lead. *Nutr Rep Int* 1989;**40**:1165–1172.
109. Chorus I, Bartram J (editors): *Toxic cyanobacteria in water*. Great Britain: WHO, E&FN Spon, 1999.
110. Rellán S, Osswald J, Saker M, Gago-Martinez A, Vasconcelos V. First detection of anatoxin-a in human and animal dietary supplements containing cyanobacteria. *Food Chem Toxicol* 2009;**47**:2189–2195.
111. Iwasa M, Yamamoto M, Tanaka Y, Kaito M, Adachi Y. *Spirulina*-associated hepatotoxicity. *Am J Gastroenterol* 2002;**97**:3212–3213.
112. Mazokopakis EE, Karefilakis CM, Tsartsalis AN, Milkas AN, Ganotakis ES. Acute rhabdomyolysis caused by *Spirulina* (*Arthrospira platensis*). *Phytomedicine* 2008;**15**:525–527.
113. Kraigher O, Wohl Y, Gat A, Brenner S. A mixed immunoblistering disorder exhibiting features of bullous pemphigoid and pemphigus foliaceus associated with *Spirulina* algae intake. *Int J Dermatol* 2008;**47**:61–63.