

## Health Beneficial Properties of Spirulina in Preventing Non-Communicable Diseases - The Green Metabolic Regulator from the Sea

(Manfaat Kesehatan Spirulina dalam Mencegah Penyakit Tidak Berjangkit - Pengatur Metabolik Hijau dari Laut)

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### ABSTRACT

*Spirulina is a type of cyanobacteria containing various micro- and macronutrients and has been used as a functional food. Due to its nutritional components, spirulina possesses antioxidant and anti-inflammatory properties, which can potentially prevent non-communicable diseases (NCDs) triggered by inflammation and oxidative stress. This review aims to provide an overview of the effects of spirulina supplementation on NCDs derived from cellular, animals, and human studies. The current literature generally showed that spirulina could protect against NCDs, such as metabolic disorders, osteoporosis, gastric ulcer, hepatic, renal, and neurological disorders. These beneficial effects were mediated through the antioxidant and anti-inflammatory effects of spirulina and seem to be dependent on dose and duration of treatment. Despite the abundance of preclinical studies, human clinical trials validating the effects of spirulina on NCDs are lacking. These preclinical findings warrant a proper clinical trial to evaluate the efficacy and safety of spirulina supplementation in protecting human against NCDs.*

*Keywords: Blue-green algae; cyanobacteria; energy metabolism; inflammation; non-communicable diseases; oxidative stress*

### ABSTRAK

*Spirulina ialah sejenis sianobakteria yang mengandungi pelbagai nutrisi mikro serta makro dan telah digunakan sebagai makanan fungsian. Disebabkan oleh komponen nutrisinya, spirulina mempunyai ciri-ciri antioksidan dan anti-keradangan yang berpotensi untuk mencegah penyakit tidak berjangkit yang diaruh oleh keradangan dan tekanan oksidatif. Ulasan ini bertujuan memberi gambaran tentang kesan suplementasi spirulina ke atas penyakit tidak berjangkit yang diterbitkan daripada kajian sel, haiwan dan manusia. Kajian kepustakaan menunjukkan spirulina boleh mencegah penyakit tidak berjangkit seperti ketidak-normalan metabolik, osteoporosis, ulser gastrik, penyakit hati, ginjal dan neurologi. Manfaat ini dijana melalui kesan antioksidan dan anti-keradangan spirulina. Walaupun terdapat banyak kajian praklinikal, kajian manusia secara klinikal masih tidak mencukupi. Keputusan kajian praklinikal ini perlu disahkan melalui percubaan klinikal yang wajar untuk menguji keberkesanan dan keselamatan suplemen spirulina dalam melindungi manusia daripada penyakit tidak berjangkit.*

*Kata kunci: Alga biru-hijau; keradangan; metabolisme tenaga; penyakit tidak berjangkit; sianobakteria; tekanan oksidatif*

### INTRODUCTION

Non-communicable diseases (NCDs) are chronic disorders, which are not transmittable from person to person, have a long duration of development and progress slowly (Ebrahim et al. 2013). Some of the NCDs include cardiovascular diseases (heart attacks and strokes), chronic respiratory diseases (chronic obstructed pulmonary disease and asthma), metabolic (diabetes and obesity)

and musculoskeletal disorders (osteoporosis) and cancer (Ebrahim et al. 2013; National Centre for Biotechnology Information 2020). NCDs pose a significant public health challenge due to the resultant increased in medical expenses, morbidity, mortality, and economic loss, as well as reduced quality of life and labor force worldwide (Mayer-Foulkes 2011; WHO 2018, 2017). World Health Organization projected that NCDs would be responsible for 70% of the

global deaths by 2025, and 85% of these deaths will occur in developing countries (WHO 2015). Furthermore, an estimated 41 million people in low-resource countries will die from NCDs by 2025 if proper prevention approaches are not designed and applied (WHO 2014a). Approximately 40% of the 38 million deaths linked to NCDs yearly are premature and preventable (WHO 2014b).

Oxidative stress has been implicated in the onset of NCDs through increased formation of intracellular oxidised molecules. Oxidative stress hinders protein folding and causes fragmentation and loss of protein function (Delanghe et al. 2017). Oxidised proteins are identified and broken down by the cells, but an excess of protein oxidation leads to the accumulation of toxic products, which cause cellular dysfunction (Delanghe et al. 2017). The build-up of these oxidised biomolecules has been linked to the onsets of various NCDs like cancer, diabetes and cardiovascular diseases (Sies 2015). Studies have reported increased levels of proinflammatory cytokines in adipose tissue, muscle, brain, liver and heart of animal models and humans affected by NCDs. The levels of these pro-inflammatory cytokines tally with the severity of the NCDs (Hartman & Frishman 2014; McLaughlin et al. 2017; Thaler et al. 2012; Wang & Nakayama 2010). For instance, increased neuronal inflammation in the central nervous system (Zhang et al. 2008) and pro-inflammatory cytokines-producing immune cell infiltration were observed in the peripheral adipose tissue in type 2 diabetes mellitus (Crewe & Scherer 2017). Increased pro-inflammatory cytokine levels have also been linked to initiation, promotion and progression of tumours (Lin & Karin 2007). Therefore, oxidative stress and chronic inflammation are two essential mediators for the initiation and progression of NCDs.

Normally, oxidative stress is regulated by antioxidants, which are divided into two groups. The first group are intracellular antioxidants, such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), which prevent free radical formation. The second group are obtained exogenously through food, for example, vitamin C, E, and beta-carotene (August et al. 1996; Stahl & Sies 1997). Chronic oxidative stress impairs the antioxidative defence of the body. For instance, oxidative stress induced by obesity may be responsible for the irregular production of adiponectin which causes metabolic syndrome (Esposito et al. 2006). Hyperglycemia in diabetic patients has been reported to incapacitate antioxidant defence system, which increases cell protein damage and diabetic complications (Pihl et al. 2006). In line with these observations, replenishing the exogenous antioxidants through diet could prevent further damage by oxidative

stress. Several epidemiological studies and intervention trials have reported that dietary antioxidants are effective against chronic diseases (Bouayed & Bohn 2010). Several meta-analyses and cohort studies have reported a link between increased consumption of antioxidant-rich foods and decreased risk of NCD-related mortality, cardiovascular diseases, and certain cancers (Grosso et al. 2017a, 2017b; Hooper et al. 2008). Furthermore, antioxidants from grape seeds, cocoa, moringa leaves, coffee, mulberry fruit, and brown rice have been reported to prevent NCDs (Ma & Zhang 2017; Ramos et al. 2017; Ravichanthiran et al. 2018; Vergara et al. 2017; Yamagata 2018; Zhang et al. 2018).

Marine organisms contain several bioactive compounds considered to be potential therapeutic agents for the prevention and treatment of NCDs (Collins et al. 2016; Pangestuti & Kim 2011). One of the candidate organisms is spirulina, a type of minute, spiral, filamentous marine cyanobacteria capable of photosynthesis. Spirulina is taxonomically designated to two main species of cyanobacteria, *Arthrospira platensis* found in Africa and Asia and *Arthrospira maxima* found in California and Mexico. Both have been utilised as dietary and feed supplements (Jung et al. 2019; Siva et al. 2015). However, the common name, spirulina, refers to the dried biomass of *Arthrospira platensis* that includes Cyanobacteria and Prochlorophyta (Gershwin & Belay 2007). Spirulina contains phycocyanin, a pigment which gives its characteristic blue-green colour (Sihna et al. 2018). It has been used as a functional food or nutraceutical because it contains proteins, vitamins, minerals, essential fatty acids, polysaccharides, glycolipids, sulfolipids, enzymes, and several pigments (Blinkova et al. 2001; Campanella et al. 1999; Cicero et al. 2017; Colla et al. 2004; Earthrise 2006; Khan et al. 2005). Besides, spirulina has been reported to possess antioxidant effects *in vivo*. Thus, spirulina may have a role in preventing or treating NCDs. This review aims to summarise the evidence of spirulina as a functional food against NCDs.

#### SPIRULINA AND METABOLIC SYNDROME (METS)

Metabolic syndrome (MetS), which is a collection of conditions, such as dyslipidemia (increased serum triglycerides and reduced high-density lipoproteins (HDL)), increased arterial blood pressure (BP), dysregulated glucose homeostasis and abdominal obesity, increases the risk of developing coronary heart disease, other forms of cardiovascular atherosclerotic disorders and Type 2 diabetes (Kassi et al. 2011; Kaur 2014). Depending on age, sex, race, ethnicity and definition of MetS, the global

prevalence of MetS varies from 10-84% (Alberti et al. 2006). The global adult population believed to be affected by MetS ranges from 20-25% (Ranasinghe et al. 2017).

The potential of spirulina as an agent to prevent MetS and its components has been investigated (Yousefi et al. 2018a, 2018b). A study by Lee et al. (2017) reported that rat pancreatic  $\beta$ -cell line (RINm5F) treated with spirulina (0.5-2.5  $\mu\text{g/mL}$ ) showed increased cell viability. Spirulina reduced the expression of IL-15, interferon gamma-induced protein 10 and CCAAT/enhancer-binding protein alpha (C/EBP) homologous protein, and inhibited C-Jun N-terminal kinase and p38 activation in RINm5F cells, indicating protection from cytokine-induced cell death. Sarcoendoplasmic reticulum  $\text{Ca}^{2+}$ -ATPase 2b expression was also upregulated in RINm5F cells cultured with spirulina, reflecting an inhibition in the loss of differentiated  $\beta$ -cell functions, reduced endoplasmic reticulum (ER) calcium depletion and ER stress (Lee et al. 2017).

In a study by Seo et al. (2018), mouse 3T3-L1 preadipocytes and C3H10T1/2 cells treated with spirulina (0-200  $\mu\text{g/mL}$ ) showed reduced lipid droplet accumulation. A downregulation in adipogenic proteins (C/EBP, proliferator-activated receptor gamma (PPAR $\gamma$ ) and adipocyte protein 2) was observed in spirulina-supplemented cells. In obesity, an increase in adipogenic proteins signifies hyperplasia (cell number increase) and hypertrophy (cell size increase) in white adipose tissue. Therefore, a downregulation in adipogenic proteins caused by spirulina suggests a decrease in the size and number of white adipose tissue. Spirulina supplementation also reduced the production of triglycerides and expression of fatty acids and triglycerides synthetic proteins by suppressing lipogenic proteins (sterol regulatory element-binding protein (SREBP)-1, acetyl-CoA carboxylase (ACC), fatty acid synthase (FAS), lysophosphatidic acid acyltransferase, lipin1, and diacylglycerol acyltransferases-1) (Seo et al. 2018).

Most animal studies have also provided evidence on the positive effect of spirulina in managing components of MetS. Several studies which utilised streptozocin (STZ), alloxan, and dexamethasone-induced animal models reported increased SOD, CAT, GPx, GST, and GSH in blood liver, kidney and bone following spirulina supplementation (Aissaoui et al. 2017; Devesh et al. 2012; Gargouri et al. 2016a; Gupta et al. 2010; Layam & Reddy 2006; Muthuraman et al. 2009; Nasirian et al. 2018, 2017; Sadek et al. 2017; Simon et al. 2018; Rodríguez-Hernández et al. 2001). These antioxidant enzymes help to catabolise free radicals, leading to reduced oxidative stress. The reduction of oxidative stress was correlated with

increased insulin level, indicative of improved survival and function of  $\beta$ -cells (Hurrell & Hsu 2017). Renal and liver profiles in the animals supplemented with spirulina were improved, denoting that hyperglycemia-induced liver and renal damages were prevented. Dexamethasone-induced insulin-resistant rats supplemented with 500 mg/kg spirulina daily for 21 days showed reduced serum low-density lipoprotein (LDL) and triglyceride levels and increased HDL levels (Devesh et al. 2012; Gupta et al. 2010). The authors attributed the hypolipidemic effects of spirulina to the presence of 5-6% of essential fatty acids which could prevent the build-up of fat and cholesterol (Grattan 1989; Kurdikeri 2006).

The metabolic effects of spirulina might be dependent on the dose and duration of treatment in studies using animals with STZ-induced diabetes. For example, in the studies by Nasirian et al. (2018, 2017), 35-day spirulina supplementation showed no effect at 10-15 mg/kg but was effective at 20-30 mg/kg. However, 45-day spirulina supplementation at 5-15 mg/kg produced more significant metabolic effects than 15 mg/kg (El-baz et al. 2013; Layam & Reddy 2006). Also, spirulina treatment at higher doses was effective in short periods. In studies by Lee et al. (2017) and Simon et al. (2018), spirulina treatment at 200 and 500 mg/kg showed significant protective effects within 4 weeks.

Rats fed with high-fat-high-sucrose (HFHS) diet is a model of MetS because they develop all conditions related to MetS (Wong et al. 2018a, 2018b, 2018c; Yang et al. 2012). HSHF diet triggers insulin insensitivity and hyperinsulinemia, prompting the progression of insulin resistance and glucose intolerance (Williams et al. 2014; Wolf et al. 2014). Wan et al. (2019) reported that spirulina (150 mg/kg daily for 8 weeks) suppressed circulating glucose level in rats fed with HFHS diet. Spirulina (150 and 450 mg/kg/day for 6 weeks) also resulted in smaller adipose depots and lower blood lipid concentrations in high-fat diet (HFD)-induced obese mice. The lower body mass gain observed in spirulina-treated mice was associated with lower protein expression of adipogenesis factors and higher expression of adenosine monophosphate-activated protein kinase (AMPK)-induced adipose browning proteins (PR domain containing 16 (PRDM16), PPAR $\gamma$  coactivator-1, and uncoupling protein-1) (Seo et al. 2018).

In another study by Heo and Choung (2018), HFD-induced obese rats treated with spirulina (62.6, 125 and 250 mg/kg, respectively, for 4 weeks) showed increased expression of phosphorylated AMPK, leading to increased expression of phosphorylated ACC (p-ACC) and decreased expression of SREBP1 and FAS. This observation suggests a decrease in lipogenesis (fatty acid and

triglyceride synthesis) in white adipose tissue and skeletal muscle. Increased expression of adipocyte-triglyceride lipase and carnitine palmitoyltransferase 1 in spirulina supplementation were also reported, suggesting increased catabolism of store fat and fatty acid oxidation. Sirtuin 1 expression was increased in mesenteric adipose tissue and skeletal muscle of obese rats supplemented with spirulina, which was attributable to the suppression in TNF- $\alpha$  and inhibition of nuclear factor kappa-light-chain-enhancer of activated B cells (NF- $\kappa$ B) pathway. Decreased adipocyte size, TNF- $\alpha$ , leptin and increased adiponectin were also reported. The combined effect of reduced lipogenesis and increased lipolysis/lipid oxidation led to a reduction in body weight of obese rats supplemented with spirulina (Heo & Choung 2018). Another study by Cheong et al. (2010) reported reduced triglycerides, total cholesterol, LDL and increased HDL in hypercholesterolemic rabbits treated with spirulina (1 and 5% for 8 weeks). They also reported reduced aortic lipid accumulation area and reduced micro and macro-cellular fatty acid changes in the liver. These results suggest that spirulina prevents the formation of atherosclerotic plaques in the aorta, thereby allowing unobstructed blood flow. In the studies using HFD, HCD, and HFHS, the protective effect of spirulina increased as the doses increased (Cheong 2010; Heo & Choung 2018; Seo et al. 2018).

In a study by Fujimoto et al. (2012), mice with monosodium glutamate-induced MetS were treated with 5% spirulina per food weight daily for 2 weeks. Spirulina protected the liver from damage by reducing aspartate transaminase (AST) and alanine transaminase (ALT) levels. Spirulina also prevented lipid accumulation by lowering triglyceride and total cholesterol levels of the rats.

The therapeutic effects of spirulina on MetS components have been investigated in several human studies. In a study by Mani et al. (2000), non-insulin-dependent diabetes mellitus supplemented with spirulina (2 g/day for 2 months) showed reduced blood glucose level, which was attributed to the high fibre content in

spirulina that reduces the absorption of glucose and the presence of peptides and polypeptides produced during spirulina digestion, which stimulates insulin release. The authors also attributed the lower serum very-low-density lipoprotein (VLDL) and triglyceride levels observed in the supplemented group to high clearance caused by fibre and protein content of spirulina. A reduction in VLDL led to a decrease in serum LDL because most of the LDL is derived from VLDL (Mani et al. 2000). Another study by Alam et al. (2016) showed that treatment with 4.5 g of spirulina powder twice daily for 45 days reduced the fasting and postprandial blood glucose of patients with type 2 diabetes mellitus and the effects was similar to patients treated with metformin (500 mg, twice daily for 45 days). In another study, 52 obese patients treated with 2 g spirulina daily for 12 weeks showed triglyceride and LDL levels and increased adiponectin, which suggests reduced lipid accumulation. Spirulina also reduced the risk of developing coronary heart disease by lowering the level of high sensitivity C-reactive protein in obese patients (Yousefi et al. 2018a).

In patients with systemic arterial hypertension, spirulina supplementation (4.5 g daily for 12 weeks) caused increased CAT, SOD, glutathione reductase (GR) and GPx activities, reduced systolic blood pressure and reduced expression of vascular cell adhesion molecule, E-selectin and endothelin-1. These observations suggest that spirulina, through its antioxidant activity, reduced the ROS-stimulated vascular endothelial associated with hypertension (Martínez-Sámano et al. 2018). Miczke et al. (2016) reported a decrease in systolic blood pressure, diastolic blood pressure and arterial stiffness index in spirulina-treated hypertensive patients (2 g daily for 3 months), which suggests reduced cardiovascular risk.

Overall, spirulina demonstrates antiadipogenic, hypoglycemic, hypolipidemic and hypotensive effects, which can prevent the development of MetS and its complications. These properties were related to antioxidant and anti-inflammatory activities of spirulina. An overview of the effects of spirulina against components of MetS is presented in Table 1.

TABLE 1. Evidence of Spirulina protection against MetS

Researcher	Study design	Findings
Cell culture studies		
Lee et al. (2017)	Cell line: Rat pancreatic $\beta$ -cell line RINm5F Mode of disease induction: IL-1 $\beta$ - and IFN- $\gamma$ -induced cytotoxicity Treatment: 1 $\mu$ g/mL spirulina for 24 and 48 h Control: Negative: no treatment Positive: no	$\uparrow$ Cell viability compared to the negative control $\downarrow$ Translocation of NF- $\kappa$ B p65 to the nucleus compared to the negative control $\downarrow$ IL-15, IP-10, CHOP, iNos, cytokine-induced ROS, phosphorylated JNK and p38 and $\uparrow$ SERCA2b compared to the negative control

Seo et al. (2018)	<p>Cell line: mouse 3T3-L1 preadipocytes and C3H10T1/2 cells</p> <p>Mode of disease induction: triiodothyronine and rosiglitazone induce adipocyte browning</p> <p>Treatment: 0, 6.25, 12.5, 25, 50, 100, or 200 µg/mL spirulina for 24 h</p> <p>Control:</p> <p>Negative: no</p> <p>Positive: chlorophyll a and phycoerythrin</p>	<p>↓ C/EBP, PPAR<math>\gamma</math> and aP2 expression compared to the positive control.</p> <p>↓ SREBP-1, ACC, FAS, LPAAT, Lipin1, and DGAT-1 expression compared to the positive control</p>
Animal studies		
Wan et al. (2019)	<p>Animals: 36 Wistar albino rats (130-170 g; 5-6 weeks old)</p> <p>Mode of disease induction: high-fat-high-sucrose diet-induced diabetes</p> <p>Treatment: 2 mL of 150 mg/kg spirulina ethanol extract or 150 mg/kg spirulina water extract daily for 8 weeks</p> <p>Control:</p> <p>Negative: HFHS diet alone</p> <p>Positive: no</p>	<p>↓ Fasting blood glucose level compared to the negative control</p> <p>↓ Serum glucose level in oral glucose tolerance test compared to the negative control</p>
Nasirian et al. (2018)	<p>Animal: 64 male Wistar rats (180-220 g; 2.5 months old)</p> <p>Mode of disease induction: streptozocin (STZ)-induced diabetes</p> <p>Treatment: 10, 20 and 30 mg/kg of spirulina daily for 35 days</p> <p>Control:</p> <p>Negative: 0.3 mL distilled water</p> <p>Positive: no</p>	<p>↑ SOD, GSH-Px, CAT and ↓ malondialdehyde, TNF-<math>\alpha</math>, IL-6 compared to the negative control</p> <p>↓ Triglycerides, Cholesterol, LDL-C, Glucose, AST, and ALT levels compared to the negative control</p>
Simon et al. (2018)	<p>Animal: 30 female Wistar albino rats (130 g)</p> <p>Mode of disease induction: STZ- induced diabetes</p> <p>Treatment: 400 mg/kg of spirulina daily for 28 days</p> <p>Control:</p> <p>Negative: STZ + no treatment</p> <p>Positive: 0.6 mg/kg glibenclamide</p>	<p>↓ Blood glucose level and renal function markers (creatinine, urea and uric acid) compared to the negative control</p> <p>↑ SOD, CAT, GPx, GST, GSH and ↓ in TBARS compared to the negative control</p> <p>↓ Total cholesterol, triglyceride and HbA1C levels compared to the negative control</p> <p>↑ <math>\beta</math>-cell and islets size in pancreas compared to the negative control</p>
Nasirian et al. (2017)	<p>Animal: 60 male Wistar rats (180-220g)</p> <p>Mode of disease induction: STZ-induced diabetes</p> <p>Treatment: 15 mg/kg and 30 mg/kg of spirulina daily for 5 weeks</p> <p>Control:</p> <p>Negative: STZ + no treatment (DC)</p> <p>Positive: no</p>	<p>↑ Red blood cells, white cell counts, mean corpuscular haemoglobin concentration, packed cell volume and mean cell volume at 30 mg/kg compared to the negative control</p>
Aissaoui et al. (2017)	<p>Animals: 48 male Wistar rats (10-13 weeks old; 180-200 g)</p> <p>Mode of disease induction: alloxan-induced diabetes</p> <p>Treatment: 1 mL/day of spirulina (10% w/v) for 50 days</p> <p>Control:</p> <p>Negative: alloxan + no treatment (DC)</p> <p>Positive: alloxan + 500 mg/kg metformin (D + Met) and BHT (<i>in vitro</i>)</p>	<p>↓ TBARS compared to the negative control</p> <p>↑ SOD, total antioxidant status, glutathione peroxidase and glutathione reductase compared to the negative control</p> <p>↑ DPPH and ABTS levels compared to positive control BHT</p> <p>↓ Hyperglycaemia and liver function markers (GPT, GOT, Alk-p) compared to the negative control</p> <p>↑ Insulin levels and ↓ HOMA-IR compared to the negative control</p> <p>↓ Cell necrobiosis and ↑ islet size in pancreas compared to the negative and positive control</p>

Sadek et al. (2017)	<p>Animal: 40 male Albino rats (180-210 g)  Mode of disease induction: STZ-induced diabetes  Treatment: 500 mg/kg of spirulina twice weekly for 2 months  Control:  Negative: STZ + no treatment  Positive: no</p>	<p>↓ Serum glucose level compared to the negative control  ↑ Serum insulin levels and ↓ HbA1c% compared to the negative control  ↑ SOD, CAT, GST and GSH activities compared to the negative control</p>
Lee et al. (2017)	<p>Animals: 36 specific pathogen-free male Wistar rats (5-6 weeks old)  Mode of disease induction: STZ-induced diabetes  Treatment: 200 mg/kg of spirulina daily for 4 weeks (pre- and post-treatment)  Control:  Negative: STZ + no treatment  Positive: no</p>	<p>↓ Blood glucose, total cholesterol, and triglyceride levels compared to the negative control  ↓ GOT and ALP compared to the negative control  ↓ Degenerative and necrotic changes of β-cells and ↑ islet size compared to the negative control</p>
Gargouri et al. (2016a)	<p>Animals: 30 Wistar rats (180-200 g)  Mode of disease induction: alloxan-induced diabetes  Treatment: 50 g of spirulina/kg of food pellet for 21 days  Control:  Negative: alloxan + no treatment  Positive: alloxan + insulin (0.5 UI/rat)</p>	<p>↓ Hyperglycaemia compared to the negative control  ↓ The activity AST, ALT, ALP, as well as T-bilirubin and D-bilirubin levels  ↓ TG, TC, LDL and ↑ HDL  ↓ TBARS, SOD levels and ↑ CAT, GPx levels compared to the negative control</p>
El-Baz et al. (2013)	<p>Animals: 60 male Wistar albino rats (200-300 g)  Mode of disease induction: STZ-induced diabetes  Treatment: 15 mg/kg of ethanol extract of <i>Spirulina platensis</i> for 45 days  Control:  Negative: DM + no treatment  Positive: DM + 5 mg/kg glibenclamide for 45 days</p>	<p>↓ Blood glucose level, liver function enzymes (AST, ALT, ALP), lipid profile (TC, TG, LDL), NO, malondialdehyde, phosphoenol pyruvate carboxykinase compared to the negative control  ↑ SOD, GSH, total protein, LDH, pyruvate kinase and hexokinase compared to the negative control  ↓ Urea and creatinine compared to the negative control</p>
Devesh et al. (2012)	<p>Animal: 45 male albino Wistar rats (180-200 g)  Mode of disease induction: dexamethasone-induced insulin resistance  Treatment: 500 mg/kg spirulina daily for 21 days  Control:  Negative: DM  Positive: DM + 10 mg/kg/day pioglitazone (DM + R)</p>	<p>↓ Fasting blood glucose, triglycerides and LDL; ↑ Insulin and HDL compared to the negative control</p>
Gupta et al. (2010)	<p>Animal: 30 male albino Wistar rats (180-200 g)  Mode of disease induction: dexamethasone-induced insulin resistance (DM)  Treatment: 500 mg/kg spirulina daily for 21 days  Control:  Negative: DM  Positive: DM + 10 mg/kg/day rosiglitazone (DM + R)</p>	<p>↓ Fasting blood glucose, triglycerides and LDL; ↑ Insulin and HDL compared to the negative control</p>

Muthuruman et al. (2009)	<p>Animal: 24 male albino Wistar rats (8-10 weeks old)</p> <p>Mode of disease induction: alloxan-induced diabetes</p> <p>Treatment: 10 mg/kg spirulina daily for 30 days</p> <p>Control:</p> <p>Negative: alloxan + No treatment</p> <p>Positive: no</p>	<p>↓ Blood glucose level and ↑ insulin level compared to the negative control</p> <p>↑ β-cells number and size of the islet of Langerhans in pancreas compared to the negative control</p>
Layam & Reddy (2006)	<p>Animal: 30 male albino Wistar rats (180-200 g)</p> <p>Mode of disease induction: STZ-induced diabetes</p> <p>Treatment: 5, 10 and 15 mg/kg spirulina daily for 45 days</p> <p>Control:</p> <p>Negative: No treatment</p> <p>Positive: 600 µg/kg glibenclamide</p>	<p>↓ Fasting blood glucose level compared to the negative and positive control</p> <p>↑ Plasma insulin, C-peptide and haemoglobin levels compared to the negative and positive control</p>
Rodriguez-Hernandez et al. (2001)	<p>Animals: 24 male mice and 24 female mice (25-30 g)</p> <p>Mode of disease induction: alloxan-induced diabetes</p> <p>Treatment: powdered spirulina diet with 5% spirulina daily for 4 weeks</p> <p>Control:</p> <p>Negative: alloxan + no treatment</p> <p>Positive: no</p>	<p>↓ Blood glucose in both male and female mice compared to the negative control</p> <p>↓ Serum triacylglycerol levels in female mice and ↓ liver triacylglycerol in male mice compared to the negative control</p> <p>↓ Total lipid content and TBARS in the liver of both male and female mice compared to the negative control</p> <p>↓ LDL and VLDL levels and ↑ HDL levels compared to the negative control</p> <p>↓ Multilobulillar steatosis in the liver of both male and female mice compared to negative control</p>
Fujimoto et al. (2012)	<p>Animals: 27 newborn Crj:CD-1 (ICR) male mice</p> <p>Mode of disease induction: monosodium glutamate (MSG)-induced metabolic syndrome (MetS)</p> <p>Treatment: 5% spirulina daily for 12weeks</p> <p>Control:</p> <p>Negative: MSG + no treatment</p> <p>Positive: 0.02% poiglitazone daily</p>	<p>↓ Body weight, liver: body weight ratio, ALT, leptin, TC and LDL compared to the positive and negative control</p> <p>↓ TG and AST compared to the negative control</p> <p>↓ Adiponectin compared to the positive control</p> <p>↓ Infiltration of macrophages into visceral fat and ↓ liver lipid accumulation and oxidative stress.</p> <p>↓ body weight</p>
Seo et al. (2018)	<p>Animals: 24 male mice (25-30 g)</p> <p>Mode of disease induction: high-fat diet (HFD)-induced obesity</p> <p>Treatment: 150 and 450 mg/kg daily for 6 weeks</p> <p>Control:</p> <p>Negative: no treatment</p> <p>Positive: no</p>	<p>↓ Blood glucose, body weight, TG, TC, LDL and ↑ HDL compared to the negative control</p> <p>↓ Subcutaneous and abdominal white adipose tissue compared to the negative control</p> <p>↑ PR domain containing 16, PPARγ coactivator-1, and uncoupling protein-1 expression compared to the negative control</p>
Heo & Choung (2018)	<p>Animals: 48 male Sprague Dawley rats (5 weeks old)</p> <p>Mode of disease induction: high-fat diet (HFD)-induced obesity</p> <p>Treatment: 62.6, 125 and 250 mg/kg daily for 4 weeks</p> <p>Control:</p> <p>Negative: 0.5% carboxymethyl cellulose</p> <p>Positive: 125 mg/kg green tea extract</p>	<p>↓ Body weight, hyperglycaemia, white adipose tissue index, adipocyte size, TNF-α, leptin and ↑ adiponectin compared to the negative control</p> <p>↑ p-AMPK, p-ACC, ATGL and CPT1 expression compared to the negative control</p> <p>↓ SREBP1 and FAS expression compared to the negative control</p>

Cheong et al. (2010)	<p>Study: 20 male New Zealand white rabbits (2-2.5 kg)</p> <p>Mode of disease induction: high cholesterol diet (HCD)-induced hypercholesteremia</p> <p>Treatment: 1 and 5% spirulina daily for 8 weeks</p> <p>Control:</p> <p>Negative: HCD + no treatments</p> <p>Positive: no</p>	<p>↓ TG, TC, LDL and ↑ HDL compared to the negative control</p> <p>↓ Aortic lipid accumulation area and cellular fatty changes in the liver compared to the negative control</p>
Human Studies		
Alam et al. (2016)	<p>Human study: 40 patients with type 2 diabetes mellitus (30 for the test group and 10 for control)</p> <p>Treatment: 7 g of spirulina powder twice daily for 45 days</p> <p>Control:</p> <p>Negative: no</p> <p>Positive: 500mg of Metformin twice daily for 45 days</p>	<p>↓ Fasting blood sugar and postprandial blood sugar similar to ↓ in the positive control group</p>
Mani et al. (2000)	<p>Human study: 22 patients with non-insulin dependent diabetes mellitus (11 males, 11 females)</p> <p>Treatment: 2 g of spirulina tablet daily for 2 months</p> <p>Control:</p> <p>Negative: no treatment</p> <p>Positive: no</p>	<p>↓ Blood glucose and glycated serum protein levels compared to the negative control</p> <p>↓ Triglycerides, total cholesterol and free fatty acid compared to the negative control</p> <p>↓ LDL-C, VLDL-C and HDL-C/LDL-C ratio compared to the negative control</p>
Yousefi et al. (2018a)	<p>Human study: 52 obese and overweight men and women (25 kg/m<sup>2</sup> ≤ BMI &lt; 40 kg/m<sup>2</sup>; 20-60 years old)</p> <p>Treatment: 2 g (4 × 500 mg) spirulina tablet daily for 12 weeks</p> <p>Control:</p> <p>Negative: Placebo</p> <p>Positive: no</p>	<p>↓ TG, LDL-C, LDL-C/HDL-C ratio, and hs-CRP compared to placebo</p> <p>↑ Adiponectin compared to the placebo</p>
Martinez-Samano et al. (2018)	<p>Study: 16 systemic arterial hypertension patients (13 females, 3 males)</p> <p>Treatment: 4.5 g of spirulina daily for 12 weeks</p> <p>Control:</p> <p>Negative: placebo</p> <p>Positive: no</p>	<p>↓ Endothelial damage indicators (vascular cell adhesion molecule, E-selectin and endothelin-1) compared to placebo</p> <p>↓ Systolic blood pressure compared to placebo</p> <p>↑ CAT, SOD, GR and GPx activities compared to the placebo</p>
Miczke et al. (2016)	<p>Study: 40 hypertension patients (19 females, 21 males)</p> <p>Treatment: 2 g of spirulina daily for 3 months</p> <p>Control:</p> <p>Negative: placebo-Positive: no</p>	<p>↔ BMI and weight compared to placebo</p> <p>↓ Systolic blood pressure, diastolic blood pressure and stiffness index compared to the placebo</p>

Abbreviation: ABTS, 2, 2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid; ACC, acetyl-CoA carboxylase; ALP, alkaline phosphatase; ALT, alanine transaminase; AP2, adipocyte protein 2; AST, aspartate transaminase; ATGL, adipose triglyceride lipase; BHT, butylated hydroxytoluene; BMI, body mass index; CAT, catalase; C/EBP, CCAAT/enhancer-binding protein alpha; CHOP, C/EBP homologous protein; CPT1, carnitine palmitoyltransferase I; DC, diabetic control; DGAT-1, diacylglycerol acyltransferases-1; DM, diabetes mellitus; DPPH, 2, 2-diphenyl-1-picrylhydrazyl; FAS, fatty acid synthase; GOT, glutamic-oxaloacetic transaminase; GPx, glutathione peroxidase; GPT, glutamic-pyruvic transaminase; GR, glutathione reductase; GSH, glutathione; GST, glutathione S-transferases; HbA1C, glycated haemoglobin; HbA1c%, haemoglobin percent; HCD, high cholesterol diet; HDL, high-density lipoproteins; HDL-C, high-density lipoproteins cholesterol; HFHS, high-fat-high-sucrose; HOMA-IR, homeostatic model assessment of insulin resistance; hs-CRP, high sensitivity C-reactive protein; IFN- $\gamma$ , Interferon gamma; IL-1 $\beta$ , interleukin 1-beta; IL-6, interleukin 6; IL-15, interleukin 15; iNOS, inducible nitric oxide synthase; IP-10, interferon- $\gamma$ -inducible protein 10; JNK, C-Jun N-terminal kinase; LDH, lactate dehydrogenase; LDL, low-density lipoprotein; LDL-C, low-density lipoproteins cholesterol; LPAAT, lysophosphatidic acid acyltransferase; MetS, metabolic syndrome; MSG, monosodium glutamate; NF-KB, nuclear factor kappa-light-chain-enhancer of activated B cell; NO, nitric oxide; p-ACC, phosphorylated acetyl-CoA carboxylase; p-AMPK, phosphorylated adenosine monophosphate-activated protein kinase; PPAR $\gamma$ , proliferator-activated receptor gamma; ROS, reactive oxygen species; SERCA2b, sarcoplasmic reticulum calcium ATPase 2b; SOD, Superoxide dismutase; SREBP-1, sterol regulatory element-binding protein; STZ, streptozocin; TBARS, Thiobarbituric acid reactive substances; TC, total cholesterol; TG, triglyceride; TNF- $\alpha$ , tumor necrosis factor-alpha; VLDL, very-low-density lipoprotein cholesterol



#### THE ANTI-OSTEOPOROSIS PROPERTIES OF SPIRULINA

Osteoporosis is a chronic metabolic bone disease characterised by reduced bone mass, deteriorated bone tissue, and disruption of bone microarchitecture, which leads to compromised bone strength and increased risk of fractures (NIH 2001; Sozen et al. 2017). Due to the morbidity, mortality, and health care cost associated with osteoporotic fractures, it has become a significant health problem (Wong et al. 2013). It was estimated that 21 million men and 137 million women were prone to osteoporotic fractures globally in 2010, and 55% of them were Asians (Oden et al. 2015).

Vitamin D is needed for the absorption of calcium and its deficiency is a major cause of osteoporosis (Lips & van Schoor 2011). In the study by Ekantari et al. (2017), it was observed that rats fed with vitamin D deficient diet and supplemented with spirulina (13% spirulina per weight of the diet for 8 weeks) showed higher calcium, magnesium, phosphorus and alkaline phosphatase in their serum compared to the untreated group. The femur length and diameter, as well as bone mineral density of the treated rats, were also increased due to higher mineral absorption coefficient compared to the untreated rats.

Chronic inflammation is the hallmark of MetS. As evidence, the expression of IL-6, TNF $\alpha$  and CRP is higher in patients with MetS (Christiana et al. 2016; Patel & Patel 2015). These cytokines are activators of bone resorption, and chronic inflammation in MetS would predispose a person to excessive bone loss (Walsh et al. 2006). Spirulina has been reported to alleviate osteoporosis associated with MetS. Devesh et al. (2012) and Gupta et al. (2010) reported an increase in trabecular bone thickness as well as a reduced number of resorptive pits in the femur of diabetic animals treated with spirulina (500 mg/kg/day for 21 days).

Heavy metal exposure, especially lead, can disrupt bone metabolism (Rodriguez & Mandalunis 2018). Studies have shown that lead exposure results in toxicity and apoptosis of mesenchymal stem cells, reduced vitamin D activation and calcium absorption, which subsequently led to increased bone resorption (Akbal et al. 2014; Doumouchsis et al. 2009; Fortin et al. 2012; Sharifi et al. 2011). A study by Gargouri et al. (2016b) examined the protective effects of spirulina on the skeletal development affected by prenatal exposure of lead. Pregnant and lactating rats were exposed to lead (6 g/L lead acetate) and treated with 5% spirulina enriched diet. The pups born to the treated dams were reported to have decreased plasma level of total tartrate-resistant acid phosphatase (TRAP), a marker of osteoclast number, and increased total alkaline phosphatase, a non-specific marker of osteoblast, as well as higher calcium level in the bone. These observations corresponded to a bone remodelling process favouring

bone formation, as evidenced by the increased femur length and weight of the pups of the treated dams compared to the untreated ones. Further examination showed that the pups also had increased antioxidant activity (SOD, CAT, and GPx) in their femur and reduced lead deposition in the blood and femur. These results suggest that spirulina protected pups from skeletal toxicity by primarily reducing bone resorption induced by excessive oxidative stress.

Overall, these studies showed that spirulina is a functional food with anti-inflammatory properties and high mineral content, which can ameliorate skeletal degeneration due to vitamin D deficiency, MetS, and heavy metal exposure. However, the effects of spirulina on bone in osteopenic models induced by sex hormone deficiency and glucocorticoid are yet to be demonstrated. These two models represent the major secondary causes of osteoporosis in humans (Chin & Ima-Nirwana. 2015; Walker-Bone 2012).

#### THE RENAL AND HEPATOPROTECTIVE EFFECTS OF SPIRULINA

Hepatocyte mitochondria and endoplasmic reticulum have been reported as the main sites of ROS generation in several types of liver diseases. A myriad of factors can deplete the hepatic antioxidant capacity and generate an excess of ROS, such as alcohol consumption, high-calorie diet, drug overdose, environmental pollutants and heavy metals (Jadeja et al. 2017). Similarly, the kidney is an active organ performing a variety of oxidative reactions in its mitochondria, thus making it susceptible to oxidative stress damage (Che et al. 2014; Sureshbabu et al. 2015). Increased oxidative stress in chronic kidney diseases have been linked to various mechanisms, such as uremic toxin-induced endothelial nitric oxide synthase uncoupling (Sibal et al. 2010), increased NOX activity (Ward & McLeish 2010; Yu et al. 2010) and depletion of cellular antioxidants due to dietary restrictions, diuretics use, protein energy-wasting, or decreased intestinal absorption of nutrients (Jankowska et al. 2017; Tbahriti et al. 2013).

Spirulina has been reported to be effective against liver and kidney diseases. The anti-inflammatory, antioxidant and antihepatotoxic effects of spirulina have been demonstrated in rats with D-galactosamine induced hepatotoxicity (Al-Qahtani & Binibead 2019). Spirulina (3, 6 and 9%) reduced inflammatory markers (TNF $\alpha$ , IL-6 and IL-1 $\beta$ ), decreased lipid peroxidation, and increased level of antioxidants (GR, GSH, GST, SOD, GPx, and CAT) and total protein in the liver, suggesting redox status and function of the liver was maintained. At 3%, spirulina offered partial protection while at 6 and 9%, it offered complete protection which suggests a dose-dependent

effect. In a study by Martin and Sabina (2016), spirulina ameliorated the effect of anti-tuberculosis drugs (isoniazid and rifampicin)-induced changes in the kidney of Wistar rats. Rats treated with spirulina (400 and 800 mg/kg/day) showed a reversal in kidney injury by reducing serum creatinine, urea, uric acid, and acid phosphatase levels. Kidney homogenate of the treated rats also showed reduced lipid peroxidation and increased levels of antioxidant enzymes (SOD, CAT, GPx, GST, and reduced GSH). The effects of spirulina at 400 and 800mg/kg/day were similar.

Overall, spirulina protects liver and kidney from the assaults of toxicant through its antioxidant activities.

#### THE NEUROPROTECTIVE EFFECTS OF SPIRULINA

Ischemic reperfusion injury occurs when the blood returns to tissue after blood supply restriction. When blood supply is restored, oxygen is re-introduced, leading to cellular protein, DNA and plasma membrane damage due to generation of free radicals. White blood cells carried into the reperfusion area by the returning blood also release inflammatory cytokines, like ILs and free radicals as a response to the tissue damage (Clarke & Wayne 2005; Maeda & Ruel 2015). The pro-inflammatory cytokines and increased oxidative stress could trigger microvascular dysfunction in the ischemic tissues or organs, such as the brain after stroke (Maeda & Ruel 2015). The neuroprotective effect of *Spirulina platensis* was established in adult Sprague-Dawley rats with ischemic reperfusion injury. *Spirulina platensis* (0.33% of the diet weight) for 4 weeks significantly reduced the volume of cerebral cortex infarction and increased post-stroke locomotor activity (Wang et al. 2005).

Parkinsonism is a set of neurological disorders causing movement problems, such as tremors, slow movement and stiffness, similar to those in Parkinson's disease (Hector & Gonzalez-USigli 2019). The most common symptoms of Parkinsonism are akinesia, bradykinesia, rigidity, tremor, and postural abnormalities (Baluchnejadmojarad & Roghani 2010; Fahn 2003). Increased ROS production resulting from the use of certain drugs and exposure to toxins could cause increased oxidative stress. Oxidative stress causes degeneration of dopaminergic neurons in the substantia nigra pars compacta, which leads to decreased dopamine levels in its striatal projections and other brain stem neurons, and disruption of the cerebral neuronal systems responsible for motor functions (Ahmad et al. 2005; Guo et al. 2018). Some of the studies suggest the use of nutraceuticals as natural antioxidants as an appropriate strategy to

diminish the progression of neurodegenerative diseases (Koppula et al. 2012). Rats with 6-hydroxydopamine-induced Parkinsonism showed reduced ipsilateral and contralateral body rotations when treated with *Spirulina fusiform* (500 mg/kg, twice daily for 44 days) (Chattopadhyaya et al. 2015).

Overall, spirulina supplementation is an effective strategy to prevent neurological disorders mediated by oxidative stress. However, these actions are yet to be proven in human studies.

#### THE WOUND-HEALING PROPERTIES OF SPIRULINA

Wound healing is the process of restoring the integrity and function of the injured skin to the normal state. Normal wound healing response starts immediately after the tissue is wounded (Rieger et al. 2014). The process begins from the inflammation and blood coagulation, followed by proliferation and migration of dermal and epidermal cells and matrix synthesis to close up the wound. It ends in tissue remodelling and maturation stage, resulting in complete healing of the skin (Bahei-Eldin et al. 2017; Syaria et al. 2015). Delayed wound healing due to the wound conditions or systemic factors increases the risk of fungal and bacterial infection and gangrene, thereby deteriorating patient's quality of life and costing huge expenditure for the patients and the society (Olsson et al. 2019).

Wound healing properties of spirulina were studied *in vivo* using L929 human fibroblasts. The fibroblast cells treated with 1.125% spirulina incorporated wound cream showed increased cell migration, proliferation and wound closure (Gunes et al. 2017). In an *in vivo* experiment, rats with 2 cm-diameter excision skin wounds were treated with 0.2 mL spirulina extract for 12 days (Bahei-Eldin et al. 2017). Three days after wounding, the spirulina-treated group showed re-epithelisation with negligible inflammatory cells in the granulation tissue. On day 7, the spirulina-treated group showed mature differentiation in epidermis. On day 12, thick epidermis with complete re-epithelisation and hair follicles were observed. These observations suggest that spirulina increased the rate of wound closure by increasing re-epithelialisation and neovascularisation process at the wound site, attributable to its effects in increasing fibroblast proliferation and collagen synthesis.

Overall, spirulina can facilitate wound healing as evidenced in cellular and *in vitro* studies. Future studies should consider examining the effects of spirulina on complicated wounds, such as in diabetic or infected models. All non-MetS related health benefits of spirulina have been summarised in Table 2.

TABLE 2. Other health benefits of spirulina

Researcher	Study design	Findings
<b>Anti-Osteoporotic Properties of Spirulina</b>		
Ekantari et al. (2017)	<p>Study: 30 male albino Sprague Dawley rats (2 months old, 100-150 g)</p> <p>Mode of disease induction: vitamin D deficient diet (DVD)</p> <p>Treatment: 83.23 g of spirulina in 632.33 g of DVD in 8 weeks</p> <p>Control: Negative: DVD Positive: 1.09 g calcium carbonate in 578.50 g DVD</p>	<p>↑ Calcium, magnesium and phosphorus compared to the negative and positive control</p> <p>↓ Alkaline phosphorus compared to the negative and positive control</p> <p>↑ Femur length, absorption coefficient and bone mineral density compared to the negative and positive control</p>
Gargouri et al. (2016b)	<p>Study: 32 pregnant female Wistar rats (7-8 weeks old; 170-180 g)</p> <p>Mode of disease induction: lead-induced toxicity (Pb)</p> <p>Treatment: 5% enriched spirulina diet for 21 days</p> <p>Control: Negative: Pb + no treatment Positive: no</p>	<p>↑ Femur length and weight compared to negative control in newborn pups</p> <p>↓ Lead concentration in bone compared to negative control in newborn pups</p>
Devesh et al. (2012)	<p>Animal: 45 male albino Wistar rats (180-200 g)</p> <p>Mode of disease induction: dexamethasone-induced insulin resistance (DM)</p> <p>Treatment: 500 mg/kg spirulina daily for 21 days</p> <p>Control: Negative: DM Positive: DM + 10 mg/kg/day pioglitazone (DM + R)</p>	<p>↑ Trabecular bone thickness, bone strength, intactness and integrity of the bone surface compared to the positive and negative control</p>
Gupta et al. (2010)	<p>Animal: 30 male albino Wistar rats (180-200 g)</p> <p>Mode of disease induction: dexamethasone-induced insulin resistance (DM)</p> <p>Treatment: 500 mg/kg spirulina daily for 21 days</p> <p>Control: Negative: DM Positive: DM + 10 mg/kg/day rosiglitazone (DM + R)</p>	<p>↓ Number and depth of resorptive pits on the surface of the bone compared to the negative and positive control</p> <p>↑ Intactness and integrity of the bone surface</p> <p>↑ Bone strength compared to the negative and positive control</p>
<b>Renal and Hepatoprotective Effects of Spirulina</b>		
Martin & Sabina (2016)	<p>Animal: 36 albino Wistar rats (127-149 g)</p> <p>Mode of disease induction: anti-tuberculosis drugs (isoniazid and rifampicin)-induced nephrotoxicity</p> <p>Treatment: 400 mg/kg and 800 mg/kg spirulina daily for 28 days</p> <p>Control: Negative: nephrotoxicity + no treatment Positive: 25 mg/kg/day of silymarin</p>	<p>↓ Creatinine, urea, uric acid and ↑ total protein compared to the negative control</p> <p>↑ Albumin and ↓ acid phosphatase compared to the negative and positive control</p> <p>↑ CAT, SOD, GST and reduced GSH compared to the negative control</p> <p>↑ GPx compared to the negative and positive control</p> <p>↓ LPO compared to the negative control</p>
Al-Qahtani et al. (2019)	<p>Animal: 36 female albino Wistar rats (5-6 weeks old)</p> <p>Mode of disease induction:</p> <p>Treatment: 3, 6, and 9% spirulina daily for 1 week</p> <p>Control: Negative: hepatotoxicity + no treatment Positive: 0.5% butylated hydroxytoluene (BHT) diet</p>	<p>↓ TNF-<math>\alpha</math>, IL-1B, IL-6 and ↑ total protein compared to the negative control</p> <p>↑ Albumin and ↓ total protein compared to the negative and positive control</p> <p>↑ CAT, SOD, GST, reduced GSH, GPx and ↓ TBARS compared to the negative control</p>

Neuroprotective Effects of Spirulina		
Wang et al. (2005)	Animal: adult male Sprague-Dawley rats (5-6 weeks old) Mode of disease induction: middle cerebral arterial ligation and reperfusion Treatment: 0.33% spirulina daily for 4 weeks Control: Negative: no treatment Positive: 2% blueberry and spirulina diet	↓ Volume of cerebral cortex infarction and ↑ post-stroke locomotor activity compared to the negative control ↓ Cortical infarction compared to the negative control ↓ Caspase -3 activity compared to the positive and negative control
Chattopadhyaya et al. (2015)	Animal: 112 Adult male albino Wistar rats (7-8 weeks old) Mode of disease induction: 6-hydroxydopamine-induced Parkinsonism Treatment: 500 mg/kg spirulina daily for 30 days Control: Negative: 6-hydroxydopamine + no treatment Positive: 20 mg/kg amantadine daily	↓ Ipsilateral and contralateral body rotations control and ↑ muscle coordination compared to the negative control ↓ TBARS and ↑ reduced GSH compared to the negative control ↓ Dopamine levels compared to the negative control
Wound Healing Properties of Spirulina		
Gunes et al. (2017)	Cell line: HS2 keratinocyte cells and L929 fibroblasts Mode of disease induction: teflon bar scratches Treatment: 0.5% and 1.125% spirulina skin creams for 10 days Control: Negative: no treatment Positive: cream without spirulina	↑ Wound closure diameter, wound closure percentage and collagen I immunoreactivity compared to the negative and positive control
Bahei-Eldin et al. (2017)	Animal: 72 adult male albino rats (180-200 g) Mode of disease induction: 2-cm excisional skin wound Treatment: 0.2 mL spirulina daily for 12 days Control: Negative: no treatment Positive: 0.2 mL carboxymethyl cellulose daily	↓ Blood vessel, macrophage and fibroblast count compared to the positive and negative control ↑ Area % of collagen fibre deposition compared to the positive and negative control ↑ Re-epithelisation compared to the negative and positive control

Abbreviation: BHT, butylated hydroxytoluene; CAT, catalase; DM, diabetes mellitus; DVD, vitamin D deficient diet; GPx, glutathione peroxidase; GR, glutathione reductase; GSH, glutathione; GST, glutathione S-transferases; IL-1 $\beta$ , interleukin 1 beta; IL-6, interleukin 6; LPO, lipid peroxidation; NO, nitric oxide; Pb-lead; SOD, superoxide dismutase; TBARS, thiobarbituric acid reactive substances; TC, total cholesterol; TG, triglyceride; TNF- $\alpha$ , tumor necrosis factor-alpha

**SAFETY CONCERNS OF SPIRULINA SUPPLEMENTATION**  
Spirulina is a type of cyanobacterium, and some cyanobacteria are well-known for their production of toxins, like microcystins, and  $\beta$ -methylamino-L-alanine known to cause gastrointestinal disturbances, and in the long term, liver damage. Contamination with microcystins has been reported in some spirulina supplements, although at levels lower than the set limit by the Oregon Health Department (de Figueiredo et al. 2004; Roy-Lachapelle et al. 2017). The U.S. National

Institutes of Health has described spirulina supplements as 'probably safe', on the condition that they are free of microcystin contamination, but 'unlikely safe' (particularly for children) if contaminated (US National Institutes of Health 2017). As organisms can be a source of toxins, anti-nutrients or other potentially harmful compounds, it is necessary to conduct toxicity testing before ingesting them (Howlett et al. 2003). According to Gutiérrez-Salmeán et al. (2015), 800 mg/kg of spirulina administered to rats (oral and single treatment) in an acute toxicity study

showed no mortality, alterations in body weights, tissues and organs. In addition, there was no allergic skin reaction with an application of up to 2000 mg/kg. Spirulina doses of 10 to 19 g per day over several months in humans have been used safely. Moreover, evidence shows that consistent spirulina ingestion in several regions of Africa up to 40 g gives rise to no adverse effects (Jung et al. 2019). Overall, as with any other processed food, strict manufacturing protocols of spirulina-based products must be enforced to ensure public safety.

#### CONCLUSION

The evidence so far suggested that spirulina is a natural supplement with few side effects. Most studies reviewed approve its therapeutic effect against NCDs through alleviating inflammation and oxidative stress and improving antioxidant status. Most other studies also reported that the therapeutic effect of spirulina might be dependent on dose or duration of treatment. Although many animal studies have been conducted to ascertain the effects of spirulina against NCDs, there is a lack of human clinical trials to validate its efficacy. Hence, well-planned human clinical trials should be conducted to determine the effectiveness of spirulina against components of NCDs.

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