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Research Paper

C-Phycocyanin: A phycobiliprotein from *Spirulina* with metabolic syndrome and oxidative stress effects

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ABSTRACT

Spirulina maxima is a cyanobacterium considered a "superfood" due to its metabolites and nutrients content. These include a complex mixture of minerals, vitamins, fatty acids, proteins, and accessory pigments. In recent years, it has positioned itself as a promising source of bioactive molecules for the treatment of several diseases, including metabolic syndrome, coronary diseases, cancer, and the improvement of health modulating oxidative stress. C-Phycocyanin is a photosynthetic pigment from green-blue cyanobacterium and the most abundant phycobiliprotein in the Spirulina genus with various pharmacological properties attributed due to its antioxidant capacity but has no one specific cellular target. This has made it a molecule of great interest in biomedical research. This review focuses on the pharmacological effects and the benefits on metabolic syndrome and oxidative stress of C-Phycocyanin.

Key words: *Spirulina,* antioxidant effects, super food, C-Phycocyanin, oxidative stress, metabolic syndrome.

INTRODUCTION

Microalgae have been consumed since ancient times by various peoples around the world, with records of consumption in the pre-Hispanic Mexica culture in Mexico. According to Bernal Díaz del Castillo, he reports that the inhabitants of Lake Texcoco collected an edible sludge in canoes that they called Tecuitlatl, to later commercialize it among the inhabitants in the form of bread or cakes. The Mexicas, lacking riding animals like horses, had a system of royal messengers called Painani, who traveled up to 300 kilometers to deliver religious or military information; these messengers were fed Spirulina collected from lakes (Ramírez and Olvera, 2006). Other Mexican pre-Hispanic cultures like the Mayas, Texcocans, Tlaxcalans and Toltecs knew the benefits of consuming this cyanobacterium and it is believed it was the basis for the exponential development of towns of up to two million inhabitants, even when the conditions for the development of agriculture were not favorable in the Valley of Mexico (Ponce López, 2013). *Spirulina* is an open-helix filamentous cyanobacterium with characteristic multicellular cylindrical trichomes. It is endemic in tropical or sub-tropical alkaline lakes with alkaline pH values and high salinity, mainly carbonates and bicarbonates. The genus *Spirulina* has several species among which *S. fusiformis, S. laxissima, S. subsalsa, S. lonar, S. labyrinthiformis, S. maxima* and *S. platensis* can be mentioned; however, the last two predominate and are marketed in Africa, Asia, and South America, as well as part of North and Central America (Pittman et al., 2011).

Spirulina has been approved as a safe food and has received GRAS (Generally Recognized as Safe) certification from the FDA (Food and Drug Administration) (Marles et al., 2011). *S. platensis, S. maxima* and *S. fusiformis* are the most studied species based on their high nutritional value. These cyanobacteria contain a rich content of macronutrients, including all essential amino acids, polyunsaturated fatty acids ω-3 and ω-6, phenolic compounds, and B-complex vitamins. On the other hand, it has an outstanding complex of accessory pigments, among which are β-carotene, α-tocopherol, xanthophylls, chlorophylls and phycobiliproteins, which contains C-

Table 1: Chemical composition of *Spirulina.*C-PC= C-Phycocyanin, GLA= Gamma-linolenic acid, SOD= Super oxide dismutase. Modified from (5–7)**.**

Component	Amount per 3 g	Daily dose intake	Component	Amount per 3 g	Daily dose intake
Total carbohidrates	<1 g	<1	Chrome	50 µg	41
Proteins	2 g	4	Sodium	35 mg	<2
β-carotene	11.250 UI	230	Potasium	60 mg	2
Vitamin K	75 µg	94	C-PC	240 mg	-
Vitamin B12	9 µg	150	GLA	32 mg	-
Iron	7 mg	39	Chlorophyll A	30 mg	-
Magnesium	15 mg	4	Total Carotenoids	15 mg	-
Manganese	0.4 mg	20	SOD	2500 U	-

Phycocyanin (C-PC) (Table 1) (Alvarenga et al., 2011; Habib et al., 2008; Rajeshwari and Rajashekhar, 2011), giving it a wide range of metabolites with various reported pharmacological activities (Finamore et al., 2017). *Spirulina* has shown several beneficial effects when consumed for periods of 1 to 12 months, in several clinical trials. Regarding doses, they have been tested from 0.5 to 20 g per day. According to the World Health Organization, the recommended daily intake of protein for adults is 0.83 g/kg per day, and *Spirulina* contains 0.7 g/1 g of dried sample of this nutritional component, making it a good source of macronutrients with a safety and nutritional value of 0.8 g/kg per day (de la Jara et al., 2018). Various systematic reviews have been conducted on the medical applications of *Spirulina* and C-PC on immuno-modulation, inflammation, and cancer (Bannu et al., 2019; Jiang et al., 2017; Liu et al., 2016; Wu et al., 2016). C-PC is one of the most abundant phycobiliprotein in the *Spirulina* genus, and it has wide reported beneficial effects on health. Hence, this review focuses on the beneficial effects of C-Phycocyanin on metabolic syndrome and oxidative stress, through a systematically review of preclinical and clinical trials from the last 10 years.

Phycobiliproteins: Components of pharmacological importance

Phycobiliproteins are water-soluble proteins, and accessory pigments present in the cyanobacteria and blue-green microalgae involved in the photosynthetic process (Pentón-Rol et al., 2011). These proteins are organized into macromolecular complexes called phycobilisomes, which are in the membranes of the thylakoids. They are membranous structures where the luminous phase of photosynthesis occurs (Rito-Palomares et al., 2001). Phycobiliproteins are colored molecules, which give absorption in the visible regions of the spectrum. Phycobiliproteins are divided into four major groups, according to their spectroscopic properties:

1. **Phycoerythrins (PE):** They present a reddish or fluorescent orange coloration and an absorption maximum between 540-570 nm. Among the PE, two pigments can be listed: phycoerythrobilin (PUB) and phycoerythrobilin (PEB). These have 2 or 3 phycobilins (biological pigments found in cyanobacteria) in the α subunit and 3 in the β subunit.
2. **Phycocyanins (PC):** Pigments with blue coloration due to the presence of the chromophore phycocyanobilin (PCB). They have an absorption maximum between 610-620 nm. The PCs include C-Phycocyanin (C-PC) and R-Phycocyanin (R-PC) which have one chromophore in the α subunit and 2 in the β subunit.
3. **Allophycocyanins (APC):** Pigments that exhibit a purple color with a PCB chromophore. They have absorption maxima at 650-655 nm with one PCB in the α subunit and one in the β subunit.
4. **Phycoerythrocyanins (PEC):** These are pigments containing PCB and phycobilivioline (PXB), chromophores present in a few species of cyanobacteria. They show absorption maxima between 570-595 nm (Glazer, 1984).

C-Phycocyanin: A bioactive pigment

C-PC is a dark blue compound obtained from *Spirulina.* C-PC is part of the phycobilisome, a macromolecular protein complex that is responsible for employing and optimizing the use of photonic energy in the regions of the spectrum where chlorophylls absorb solar energy poorly and is used to complement the photosynthetic complex of cyanobacteria. The molecular mass, position, and intensity of the maximum absorption of C-PC depends on the state of aggregation, which is influenced by various parameters, such as the pH of the solution, temperature, protein concentration and the origin of the algae from which it comes (Mysliwa-Kurdziel and Solymosi, 2016; Wu et al., 2016). This protein carries 3 prosthetic groups, which are chromogenic molecules. These groups have a structure very similar to that of bilirubin, which is an endogenous

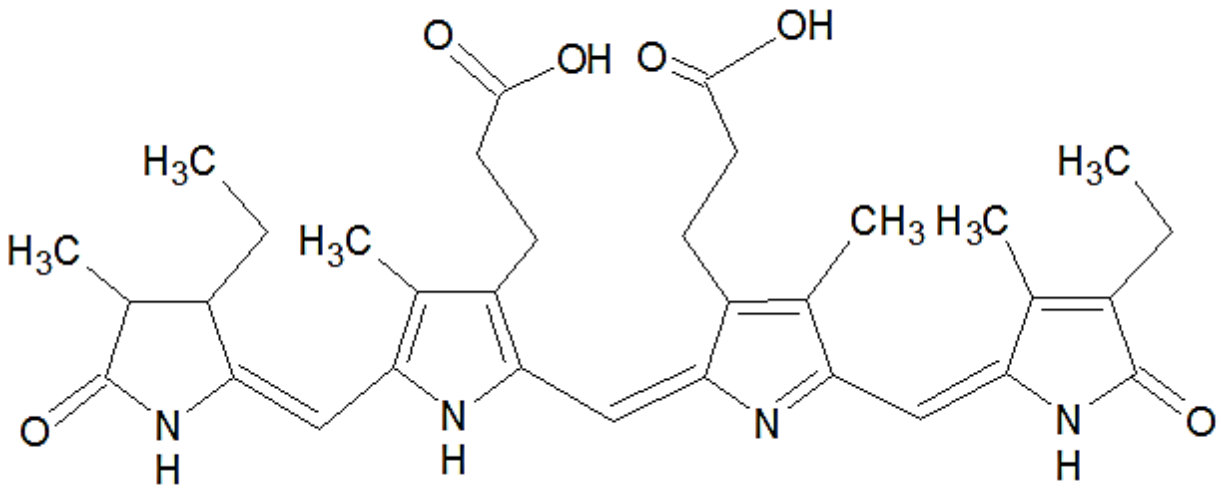

Figure 1: Structure of phycocyanobilin, the prosthetic group of the phycobiliproteins C-PC and APC. The tetrapyrrole system is formed by four rings that give rise to a compound with conjugated insaturations. PubChem CID 6438349.

molecule with known antioxidant activity. These chromophore groups inhibit microsomal lipid peroxidation because of their interaction with peroxylic radicals (Jespersen et al., 2005). Phycocyanobilin is the pigment that constitutes the prosthetic group of C-PC and APC; this corresponds to that of a system conjugated by four pyrrolic rings (Figure 1).

Pharmacological properties of C-PC

According to several studies and reviews developed, C-PC is the most abundant phycobiliprotein in *Spirulina* genus, and it has a lot of beneficial effects due to their antioxidant properties acting on multiple cellular targets. It acts on the membrane at cytoskeleton level, cytoplasm, proteins, enzymes like COX-2, and nucleus (Fernandes et al., 2018; Memije-Lazaro et al., 2018). The pharmacological properties reported in vitro and in vivo for C-PC are diverse, it acts modulating diverse endogenous biomolecules which regulate the activation of secondary signaling pathways, giving as a result biological effect. C-PC exerts antioxidant effects increasing the activity of endogenous enzymes, ameliorating deleterious effects of reactive chemical species. On the other hand, show anticancer activity modulating nuclear factors implicated in the pathogenesis. In the central nervous system, it increases the beneficial remodeling of nerves, diminishing ROS. In addition, C-PC regulates adipokines and hormones implicated in obesity and metabolic syndrome, getting better insulin sensitivity, and modulating lipids. Finally, it shows immunomodulating effects, decreasing pro-inflammatory mediators and immunity cells activity (Grover et al., 2021). These effects are summarized in Figure 2.

Effects on metabolic syndrome

In recent years, it has been proven that "superfoods" such as Spirulina can help preventing the development of pathologies such as cardiovascular disease and diabetes mellitus by preventing the development of metabolic syndrome. Its consumption has shown to help to reduce risk key-factors, such as, hypertension, dyslipidemia, insulin resistance and abdominal obesity (Bobescu et al., 2020; Van Den et al., 2018). C-PC has a wide range of cellular effects tested both, *in vitro* and *in vivo* models. Their effects on metabolic syndrome have been reported widely. It is known as the metabolic regulation activity of hydrolysates of phycobiliproteins on DPP-IV (dipeptidyl peptidase IV), affecting the regulation of peptides such as GLP-1 (incretins glucagon-like peptide 1) and GIP (gastrointestinal insulinotropic peptide). This plays important roles in response to metabolic dysfunctions such as diabetes mellitus type 2, prediabetes, and metabolic syndrome (Li et al., 2020). In metabolic syndrome, there are elevated levels of free fatty acids, insulin resistance, and decreased levels of adiponectin. This adipokine plays an important role in the regulation of lipid metabolism and insulin sensitivity. C-PC has shown beneficial effects on adiponectin expression, stimulating the production of eNOS and improving the metabolic dysregulations in this disease (Bobescu et al., 2020).

It showed a dose-dependent decrease in blood pressure, due to a correlation between endothelial nitric oxide synthase (eNOS) and the expression of adiponectin receptors and the serum levels. Because of this, C-PC may be beneficial in preventing endothelial dysfunction caused by comorbidity with high blood pressure, atherosclerosis, and development of metabolic syndrome (Ichimura et al., 2013). Pre-clinical evidence has reported that the

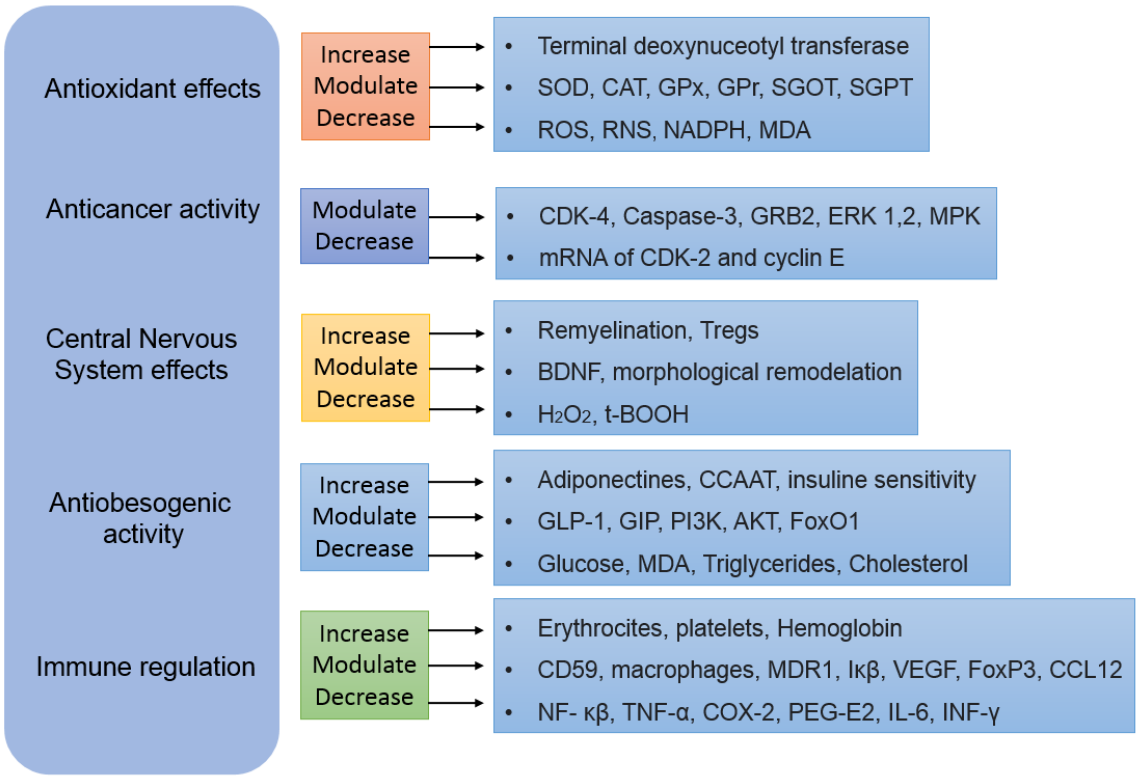

Figure 2: Main biological effects reported to C-PC.

administration of C-PC at a dose of 100 mg/kg for 3 weeks, to spontaneously diabetic KKAy mice, significantly reduces body weight, plasma glucose levels, and improves insulin sensitivity and excretion by protecting pancreatic INS-1 cells β. These changes are observed as CP-C acts by reducing malondialdehyde (MDA) levels, modulating the phosphatidylinositol 3-kinase (PI3K/Akt) and FoxO1 signaling pathway. These signaling pathways are involved in energy metabolism and metabolic regulation and are important to understand the imbalances that lead to the development and complication of diabetes and obesity (Huang et al., 2018; Peng et al., 2020). As a result, at histopathological level it was found that the administration CP-C suppresses morphological abnormalities in the islets of the pancreas like dilatation of the pancreatic conduct, cystic dilatation, and vacuolation in islet cells, inducing tissue regeneration and marked recovery of beta cells (El-Sayed et al., 2018).

Therefore, it is possible that C-PC reduces triglyceride and total cholesterol values, increasing glycogen synthesis in liver and muscle, regulating glycolipid metabolism (Ou et al., 2013; Suliburska et al., 2016). Additionally, the preventive effect of C-PC in mice administered with aloxane has been reported, finding that there is a decrease in blood glucose and glycosylated serum protein (GSP), maintains total antioxidant capacity (T-AOC), prevents MDA formation in liver, and pancreas, and induces decreases in serum and liver total cholesterol and triglycerides, increases liver glycogen levels, maintains liver glycogen kinase (GK) expression, and decreases p53 protein expression in pancreas at mRNA level (Gao et al., 2016), giving excellent properties to ameliorate the development of metabolic syndrome.

Effects on oxidative stress

Oxidative damage is caused by reactive chemical species that exerts negative effects on health. Numerous studies associate oxidative stress with chronic diseases, leading to mitochondrial dysfunction, involving a complex network of molecular and biochemical mechanisms (Shen et al., 2017). C-PC has been the subject of several pharmacological studies because of its ability to trap reactive oxygen and nitrogen species (ROS and RNS, respectively), as well as its structural similarity to biliverdin, a biliary pigment produced by hemoglobin catabolism, which is a potent inhibitor of NADPH oxidase. This multiprotein complex is the main source of ROS and oxidative imbalance at cellular level, involved in the pathogenesis of various diseases. Its inhibition could prevent the degeneration of signaling pathways, generating alterations at the level of tissues, inflammation, and immune responses of the same (Izadi and Fazilati, 2018). There have been numerous investigations at a preclinical level to study the antioxidant capacity against free radicals, and this activity has been

evaluated in vitro using the 2,2-diphenyl-1-picrylhydrazil (DPPH) assay, finding that it could prevent the consequent oxidative damage, which may explain its beneficial properties against deleterious effects of oxidative stress (Dejsungkranont et al., 2017; Fernández-Rojas et al., 2014; Manirafasha et al., 2018).

Likewise, it has been tested for its anti-radical capacity against the damage produced by tert-butyl peroxide (t-BOOH) in human SH-SY5Y neuroblastoma cell lines. It has been proven that it significantly reduces the damage caused by lipoperoxidation at 50 μM doses without observing cytotoxic effects, showing an improvement in cell viability *in vitro* and demonstrating the safety of its administration in animal models (Marín-Prida et al., 2012). C-PC has also been evaluated in ex-vivo models of perfused liver, in which it has been reported that it significantly reduces the lesions caused in the liver due to the process of ischemia and reperfusion to doses of 0. 2 mg/mL. This through reducing the activity of the enzymes alanine aminotransferase and aspartate aminotransferase (AST and ALT, respectively), alkaline phosphatase (ALP) and MDA, as well as the activity of antioxidant enzymes such as glutathione-s-transferase (GST) and glutathione peroxidase (GPx) preventing damage to the microvasculature and liver parenchyma (Gdara et al., 2018). In relation to the capacity to diminish the phenomenon of lipidic peroxidation of membranes, it has been proven that C-PC administered intraperitoneal at a dose of 50 mg/kg, has a protective potential in lung lesions induced by Paraquat toxicity (PQ) by decreasing the levels of hydroxyproline (HYP), nuclear transcription factor kappa β (NF-kβ), transcription factor inhibitor NF-kβ-alpha (Ikβ-α), and tumor necrosis factor-alpha (TNF-α), and on the other hand, glutathione peroxidase (GPX) and superoxide dismutase (SOD) activities were increased, significantly decreasing the lung damage of the treated groups (Sun et al., 2011). This strengthens the information about the properties of C-PC against oxidative stress products and lipoperoxidation, through the decrease of reactive species that could increase the mitochondrial dysfunction and the consequent development of organs malfunctions in population.

CONCLUSIONS

The pre-clinical research reported thus far provide a wide panorama about the pharmacological potential of *Spirulina* and phycobiliproteins such as C-PC against pathologies like metabolic syndrome and the deleterious effects of oxidative stress in health. Its ancestral consumption in the cultures of the Valley of Mexico allowed the maintenance and growth of cities with high population density, even when they didn't have farm animals and the food sources were reduced. The chemical composition of the cyanobacteria has made it be included within the denomination of "super food" and such properties have positioned this organism as a nutraceutical, which has been seen, can be adjuvant in pharmacological therapies. C-PC has several pharmacological effects due to its antioxidant capacity. C-PC exerts beneficial effects regulating hypertension, insulin resistance, and dyslipidemia, associated with metabolic syndrome. Its administration modulates key peptides, hormones, adipokines and activates signaling pathways involved in metabolic regulation. Finally, it reduces circulating lipids and glucose in blood, and reverts cellular abnormalities on pancreatic islets, protecting against deleterious complications of metabolic syndrome. This phycobiliprotein has proven to decrease the effects of free radicals and the consequent mitochondrial dysfunction, stimulating antioxidant enzymes like SOD and scavenging MDA. This ameliorates negative effects of lipoperoxidation and provides cellular protection against ROS and RNS, which are associated with the appearance and development of multiple chronic non-communicable diseases. It is important to develop and evaluate new sources of bioactive molecules like *Spirulina*. However, more pre-clinical and clinical trials must be carried out to provide safety and efficacy and validate its use in evidence-based medicine.

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