



## SECTION: SYSTEMATIC REVIEW AND META-ANALYSIS

## Cyanobacteria for Cardiomyocyte Protection against myocardial ischemia injury: a systematic review of animal and in vitro studies

*Cianobactérias para a Proteção de Cardiomiócitos contra Lesões Isquêmicas Miocárdicas: uma revisão sistemática de estudos em animais e in vitro*

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### Abstract

**Objective:** To perform a systematic review on using Cyanobacteria for protecting the cardiac tissue against damage caused by ischemia.

**Methods:** this review encompasses *in vitro* and controlled animal experimental studies.

**Results:** the results show that in general there are two types of interventions for treatment of ischemia and Ischemia/Reperfusion (IR) in cardiac tissue: (1) extracts treatments and (2) injection of Cyanobacteria in the damaged tissues. Extract treatments are based on the antioxidant potential of Cyanobacteria, and the studies focus mainly on Spirulina (*Arthrospira platensis*). The direct injection methods are based on the high capacity of these organisms to release oxygen during photosynthesis. *Synechococcus elongatus* is the Cyanobacteria species most commonly utilized in injections, either delivered independently or carried by hydrogels or nanoparticles. The direct Cyanobacteria injections are innovative techniques which can promote protection against apoptosis and have shown promising results, however, further research is necessary to refine the techniques and improve overall efficacy.

**Conclusion:** the effects of these treatments were beneficial considering that the antioxidant effects of Cyanobacteria ameliorate blood biochemical markers and reduce damaged cardiac areas. The oxygen releasing of Cyanobacteria in the cardiac tissue also promoted recovery of cardiac tissue after ischemia or IR.

**Keywords:** cyanobacteria, spirulina, ischemia, cardiovascular diseases.

### Resumo

**Objetivo:** realizar uma revisão sistemática sobre o uso de cianobactérias para proteção do tecido cardíaco contra danos causados pela isquemia.

**Métodos:** esta revisão abrange estudos experimentais *in vitro* e estudos controlados em animais.

**Resultados:** os resultados indicam que, em geral, existem dois tipos de intervenções para o tratamento de isquemia e isquemia/reperfusão (IR) no tecido cardíaco: (1) tratamentos com extratos e (2) injeção de cianobactérias nos tecidos danificados. Os tratamentos com extratos baseiam-se no potencial antioxidante das cianobactérias, e os estudos concentram-se principalmente em Spirulina (*Arthrospira platensis*). Os métodos de injeção direta são fundamentados na alta capacidade desses organismos de liberar oxigênio durante a fotossíntese. *Synechococcus elongatus* é a espécie de cianobactéria mais comumente utilizada em injeções, seja entregue de forma independente ou transportada por hidrogéis ou nanopartículas. As injeções diretas de cianobactérias são técnicas inovadoras que podem promover proteção contra a apoptose e mostraram resultados pro-



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missores, no entanto, mais pesquisas são necessárias para aprimorar as técnicas e melhorar a eficácia geral.

**Conclusão:** Os efeitos desses tratamentos foram benéficos, considerando que os efeitos antioxidantes das Cianobactérias melhoraram os marcadores bioquímicos sanguíneos e reduzem as áreas cardíacas danificadas. A liberação de oxigênio pelas cianobactérias no tecido cardíaco também promoveu a recuperação do tecido após isquemia ou IR.

**Palavras-chave:** cianobactérias, spirulina, isquemia, doenças cardiovasculares.

## Introduction

Ischemia is characterized by the restriction of blood flow to living tissues, caused by atherosclerotic plaques, which are formed as a result of the oxidation of cholesterol and formation of inflammatory processes in arteries. This process leads to hypoxia, depletion of metabolic substrates (such as glucose) and consequently cell damage, culminating on destruction of cell membranes (necrosis) or activating apoptosis mechanisms. Another important consequence of ischemia is the Ischemia/reperfusion (IR) injury, in which the following restoration of blood flow to previously ischemic tissues provoke significant effects, worsening cell damage and affecting also adjacent tissues (1). IR injury is a highly complex process involving tissue hypoxia, energy production through anaerobic metabolism, lactic acid accumulation, and reactive oxygen species (ROS) production as the main mechanisms. Increased ROS production and cytokine release can lead to apoptosis and necrosis (2, 3).

Cardiovascular diseases (CVD), such as atherosclerosis are the leading cause of death globally, taking an estimated 17.9 million lives each year. More than four out of five CVD deaths are due to heart attacks and strokes, and one third of these deaths occur prematurely in people under 70 years of age. Every year, nearly \$32 billion US dollars are spent on the treatment of heart failure in the United States(4). The most important behavioral risk factors of heart disease and stroke are unhealthy diet, physical inactivity, tobacco use and harmful use of alcohol (5). Furthermore, substances used in health treatments, such as Doxorubicin, a potent antineoplastic agent, can

have associated risk of developing cardiomyopathy and congestive heart failure. The cardiotoxicity of doxorubicin is also associated with oxidative stress and apoptosis (6).

Exercising and eating behavior are factors that can avoid cardiovascular diseases. Furthermore, natural compounds such resveratrol and vitamins and minerals, such as A, E, and C, and zinc may slow the development and progression of CVD (7). Currently, many studies are testing the protection effects of plant extracts for the cardiovascular system. For example, flavonoids extracted from *Apocynum venetum* L. possess various biological effects, such as lowering blood pressure levels, sedation, diuresis, anti-aging, and improving immunity (8). *Salvia miltiorrhiza* Burge (Danshen) extracts present protective actions including myocardial infarction (MI), myocardial IR injury, arrhythmia, cardiac hypertrophy, and cardiac fibrosis. These studies are mainly developed in China, related to the oriental medicine and present promising results to offer cardiovascular protection against infarction and infarction/reperfusion damage. Other innovative studies suggest the injection or the using of circulatory scaffolds combined with oxygen releasing materials or organisms, such as the Cyanobacteria *Synechococcus elongatus* Nügeli. These systems can gradually and directly supply oxygen to cardiomyocytes (CM), until new vessels take over to support metabolic needs (4).

Cyanobacteria are prokaryotic photoautotrophs considered as a great source of bioactive compounds with many biotechnological applications such as cosmetics, anticancer, antioxidant, anti-obesity, anti-inflammatory and antiviral. Also, Cyanobacteria are used as food supplement, being used as a source of proteins, especially *Arthospira platensis* Gomont (commercially called Spirulina) (9). This potential of Cyanobacteria in the treatment of ischemia and IR is due to the high photosynthetic capacity of these organisms and the presence of photosystem II, which is responsible for the oxygen release in these reactions. Cyanobacteria presents different pigments, such as, chlorophyll, carotenoids and phycobiliproteins

capable to absorb a great array of wavelengths, giving them advantage to perform photosynthesis even with low light conditions (9). Another important point is that the heart protection is due to the high antioxidant and anti-inflammatory properties of Cyanobacteria natural products. The antioxidant activities prevent ROS accumulation and cell damages caused by ischemia and IR processes. During ischemia and IR, mitochondria release elevated proportion of free radicals which exacerbate the effects of oxygen and glucose depletion in cells. This is worsened by the following inflammatory event, increasing the tissue necrosis or injury area. All these factors are responsible to various mechanisms involved in this IR injury, such as membranes injuries, DNA fragmentation and lipid peroxidation, causing necrosis (1).

Antioxidant and anti-inflammatory compounds of Cyanobacteria are well studied and play important roles in mitigating the effects of ROS and inflammation (10). For example, Spirulina, is a safe food supplement, recognized by Food and Agriculture Organization (FAO) (11), and present a variety of biological activities, including improvement of exercise performance (12) and cardiovascular protection due to its antioxidant properties (9). Although Cyanobacteria extracts or cells have shown potential for cardiovascular protection, they have been rarely directly tested on cardiomyocyte cell lines or *in vivo* experiments for assessing their effectiveness in CVD treatments. Consequently, the specific compounds responsible for heart protection and their dose-response relationships are not completely understood. Furthermore, the techniques utilized for injecting cyanobacterial cells in CVD studies need further improvement. Moreover, to our knowledge, there are no systematic reviews concerning the repercussions of the Cyanobacteria on the treatment of ischemia and IR in cardiac tissue, *in vitro* or in animal experimental studies.

Considering that, in this paper we present a systematic review of scientific papers to explore the effects of Cyanobacteria natural products and cells on cardiovascular treatment and protection against ischemia and IR in cardiac tissue, *in vitro* or in animal experimental studies.

## Methods

### Criteria for considering studies for this review

#### Types of studies

Controlled studies, that evaluated the effects of Cyanobacteria natural products and cells on cardiovascular protection against ischemia and IR, were included in the review. Reviews were excluded.

#### Types of participants

Laboratory rats of any strain, sex, weight, or age were included. Cardiac and endothelial cell lineages of any type were included. Analysis performed in other animals or tissues were excluded.

#### Types of interventions

Application of Cyanobacteria or Cyanobacteria extracts on MI damaged cardiac areas.

#### Types of outcome measures

Antioxidant effect of Cyanobacteria on CM; effects of injection of Cyanobacteria on MI injured areas.

## Search methods for identification of studies

### Data sources

A literature search for original research papers evaluating the effects of Cyanobacteria natural products and cells on cardiovascular treatment and protection against ischemia and IR was performed by searching the bibliographic databases MEDLINE (Pubmed) and ISI (Web of Science) reference lists of articles and private databases. The search was carried out by two independent reviewers (G.S.H and V.S.H) from 20<sup>th</sup> to 30<sup>th</sup> June 2023.

### Period and Language

No publication date or language restrictions were imposed.

## Search terms

We developed a literature search strategy (search terms) based on organisms used in treatments ("Cyanobacteria OR Spirulina"), and the disease (Ischemia): ((Cyanobacteria) OR (Spirulina)) AND (Ischemia).

## Data collection and analysis

### Study selection

After excluding duplicate and triplicate references, two independent reviewers (G.S.H and V.S.H.) evaluated the titles and abstracts of the identified references on the basis of inclusion and exclusion criteria. Clearly irrelevant references were excluded and all titles/abstracts that did not provide enough information about the inclusion and exclusion criteria were selected for review of the full text. In the second phase, the reviewers evaluated the full text and then selected references on the basis of the same inclusion and exclusion criteria. Moreover, one article was retrieved from these references and manually included in this review (see "Results").

## Data collection process

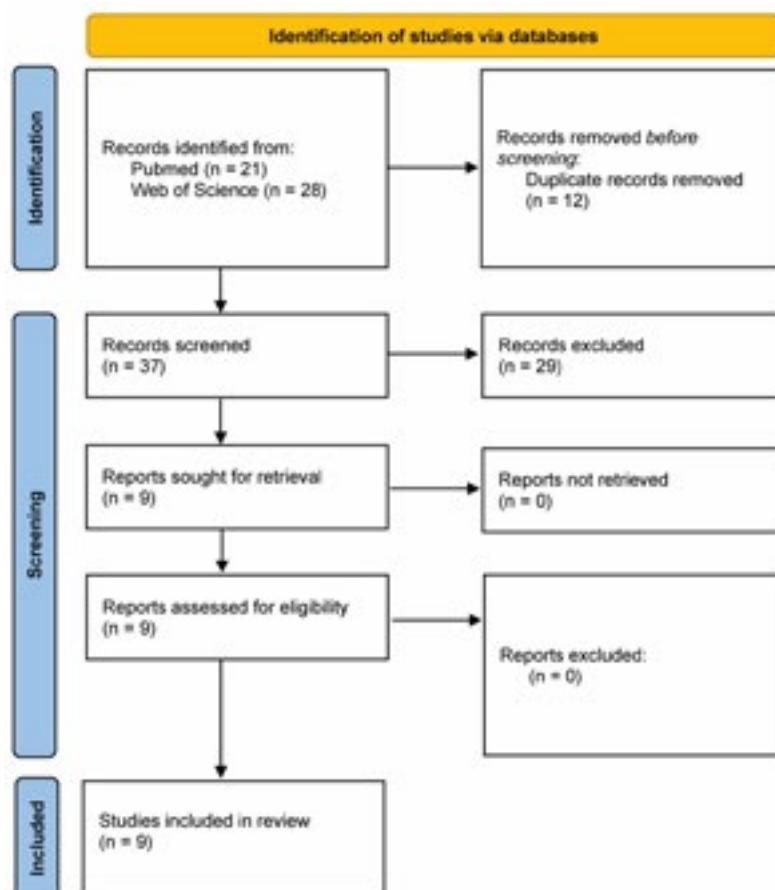
The same two independent reviewers extracted data (see "*Data items*") from each included study.

## Data items

The items extracted from each included study were the following: reference information (authors, date, journal name); objectives; experimental groups with sample size; intervention (type and dose); control groups and outcomes.

## Results

**Figure 1** shows a flowchart of search methods for identification and selection of studies. A total of 48 articles were found in the databases, of which 12 duplicates were excluded and 29 were excluded based on title, abstract, inclusion and exclusion criteria. One article was retrieved from the others references. A total of nine articles were included in this review. **Table 1** summarize the outcomes of each of the selected articles.



**Figure 1** – Flowchart of search methods for identification and selection of studies.

**TABLE 1 –** Characterization of studies using Cyanobacteria as treatment to stroke-induced damage.

Article	Objective	Population	Intervention	Control	Outcome
Strasky et al.(20)	To test the effects of <i>A. platensis</i> and PCB on the regulation of HMOX1.	<i>In vitro</i> :Human endothelial cell lines (EA.hy926) .(n=6)	-SP (0.5 g L <sup>-1</sup> ). -PCB (200 mM).	Hemin (30 mM) as positive control	-Both Upregulated Hmox1 (antioxidant effect)
		<i>In vivo</i> :Ten-week-old ApoE-deficient female mice. (n=16)	Mice fed the same control diet, but enriched with 1 g per kg bodyweight (BW) per day of freeze-dried <i>A. platensis</i> (n=8).	Mice fed with diet containing 1% of cholesterol (chow diet AIN-93M) (n=8)	-Overexpression of Hmox1 (antioxidant effect)
		<i>In vivo</i> :male transgenic mice with the transgene Hmox1 promoter driving expression of the reporter gene luciferase (Hmox1-luc). (n=16)	SP, in a dose of 0.5 g per kg. (n=8)	Animals fed with diet containing 1% of cholesterol (chow diet AIN-93M). (n=8)	-Overexpression of Hmox1 (antioxidant effect)
Sari et al.(2)	To investigate the role of melatonin and SP on multiorgan damage induced by IR in a rat model	<i>In vivo</i> :Male Wistar rats. (n=16) (abdominal aorta clamped (ischemia) and released (reperfusion))	SP (50 mg/kg). (n=8)	Distilled water. (n=8)	-Increased total antioxidant capacity and SOD levels -Decreased total oxidant status, oxidative stress index and MPO -Reduced IR-related tissue damage and apoptosis -Reduced cell swelling, focal necrosis, and neutrophil infiltration in the interstitial area and between CM
Cohen et al.(24)	To present a system that rescues the myocardium from acute ischemia, using photosynthesis through intramyocardial delivery of <i>S. elongatus</i> .	<i>In vitro</i> :CM cultures isolated from rats (light/dark; hypoxia/oxygen conditions). (n=12)	On 24-well tissue culture plates: 3 wells were plated with 500,000 cells per well along 10 <sup>7</sup> <i>S. elongatus</i> . Experiments were made in light and dark.	-3 wells plated with isolated CM at a density of 500,000 cells per well - 3 wells with 10 <sup>7</sup> <i>S. elongatus</i> alone - 3 wells with cell growth medium alone	- <i>S. elongatus</i> did not affect the survival of CM - <i>S. elongatus</i> can actively undergo photosynthesis under mammalian physiological conditions -CM cocultured with <i>S. elongatus</i> in the light trended toward higher oxygenation than did CM alone -Hypoxia-light group demonstrated a significant increase in cellular metabolism as compared to the hypoxia-dark group
		<i>In vivo</i> : male Wistar rats with induced ischemia (LAD permanently occluded). (n=20)	1 × 10 <sup>6</sup> <i>S. elongatus</i> suspended in PBS directly injected to the ischemic area. (n=5). Experiments were made in light and dark.	-PBS (n=5) - <i>S. elongatus</i> under dark conditions in animals after coronary ligation (n=5) - <i>S. elongatus</i> in animals that did not undergo coronary ligation (n=5)	-In the light, <i>S. elongatus</i> -treated hearts demonstrated a nearly 25-fold increase in oxygenation levels - <i>S. elongatus</i> group demonstrated an increase in local temperature from the time of ligation, indicating enhanced metabolic activity - <i>S. elongatus</i> treated animals enhanced ventricular contractility and overall cardiac performance
		<i>In vivo</i> : (IR):LAD was temporarily occluded. (n=17)	Injection with 1 × 10 <sup>6</sup> <i>S. elongatus</i> suspended in PBS (n=10). Experiments were made in light and dark.	Injection with PBS (n=7)	-Serum troponin significantly reduced in the <i>S. elongatus</i> -treated Group -Augmented LV ejection fraction and reduced end-systolic volume in <i>S. elongatus</i> -treated animals

**TABLE 1 –** Characterization of studies using Cyanobacteria as treatment to stroke-induced damage (cont.).

Article	Objective	Population	Intervention	Control	Outcome
Liu et al.(14)	To synthesize a hydrogel encapsulated upconversion cyanobacterium nanocapsule for both MI prevention and treatment	<i>In vitro</i> : cardiac H9C2 cells (injured by H <sub>2</sub> O <sub>2</sub> ) (n≥15)	-Incubation with culture medium containing H <sub>2</sub> O <sub>2</sub> (400 μM) (model) (n≥3) -UCCy@GelNIR (n≥3) -UCCy@Gel (n≥3) -NIR (n≥3)	No treatment, not injured (n≥3)	-Developed a hydrogel-coated upconversion cyanobacterium nanocapsule (UCCy@Gel) for MI prevention and therapy. -Photosynthesis oxygenates injured tissues after ischemia -oxygen generated under light suppressed macrophage polarization to the M1 state, reduced the levels of pro-inflammatory (TNF- and IL-6), and increased the levels of anti-inflammatory cytokines -Respiration of Cyanobacteria in dark conditions have protective effect for CM.
		<i>In vivo</i> : rats (LAD occluded) (n≥6)	-MI model (n≥3) -MI+UCCy@Gel(dark/NIR phases) (n≥3)	-LAD not ligated	
Gao et al.(6)	To study the effects of C-phycocyanin on mitochondrial dynamics of CM	<i>In vitro</i> :H9c2 cells, derived from embryonic rat heart ventricle (oxygen-glucose deprivation). (n=30)	PC (0, 10, 20, 40, 80 µg/ml) (n=5)	Not exposed to IR (n=5)	-PC treatment reduced cell death after IR -Intracellular ROS production was decreased in PC-treated groups -PC treatment ameliorated unbalanced mitochondrial dynamics induced by IR inhibiting mitochondrial fission while promoting fusion
Khan et al.(21)	To investigate the cardioprotective role of PC and SP against IR injury and the underlying signaling mechanism(s) therein	Isolate hearts kept in perfusion from Sprague-Dawley rats (Ischemia and IR) (n=32)	-Isolate hearts with Phycocyanin (10 µM) (n=8) -Isolate hearts with SP (50 mg/l) (n=8)	-Isolate hearts with no treatment (n=8) -Isolate IR hearts (n=8)	-The percent recovery of LVDP and RPP after IR was higher in hearts infused with PC or SP than in control hearts. -LDH activity decreased in coronary effluent from hearts treated with PC and SP compared to control. -CK activity decreased in coronary effluent from hearts treated with PC and SP compared to control. -PC and SP treatments reduced the infarct size -PC and SP treatments attenuates generation of free radicals during reperfusion -PC reduced the number of apoptotic cells
Bakir et al.(16)	To investigate the ability of <i>L. majuscula</i> to produce GNPs. To investigate anti-myocardial infarction activity of the produced GNPs and cyanobacterial extracts	Male Sprague-Dawley rats: acclimatation with saline injection (n=24)	N/A	-Injection with normal saline (IP) (n=6) -Cyanobacterial extract (200 mg/kg/day, IP) (n=6) -GNPs (200 mg/kg/day, IP) (n=6) -Mixture of cyanobacterial extract and GNPs (200 mg/kg/day each, IP) (n=6)	- <i>L. majuscula</i> was able to produce GNPs -Treatment with GNPs alone, and the combination of extract/GNPs ameliorated the ISO-induced escalation of the diagnostic cardiac indicator enzymes (CK, CK-MB, and cTnT), in comparison with ISO-control rats -Treatment with GNPs, and extract+GNPs prevented the ISO-induced decline of SBP, DBP and MBP -The increase in HR was attenuated in GNP and extract+GNP rats, compared to the ISO control -Treatment with GNPs and extract+GNP, showed a reverse in ECG-induced alterations presented in the ISO treated rats -Treatment with bacterial extract, GNPs, and extract+GNP, increased the activity of GRx, and SOD when compared to ISO-treatment alone
		Male Sprague-Dawley rats: acclimatation with ISO (n=24) to induce infarction (100 mg/kg, SC)	-Cyanobacterial extract (200 mg/kg/day, IP) (n=6) -GNPs (200 mg/kg/day, IP) (n=6) -Mixture of cyanobacterial extract and GNPs (200 mg/kg/day each, IP) (n=6)	-Injection with normal saline (IP) (n=6)	

**TABLE 1 –** Characterization of studies using Cyanobacteria as treatment to stroke-induced damage (cont.).

Article	Objective	Population	Intervention	Control	Outcome
Younis et al.(15)	To investigate the ability of <i>Cyanothece</i> sp., to produce GNPs and their effect on myocardial infarction injury	Male Sprague-Dawley rats: acclimatation with saline injection (n=24)	N/A	-Normal (saline injection) (n=6) -Bacterial extract injection (200 mg/kg/day, IP) (n=6) -Blue GNPs (200 mg/kg/day each, IP) (n=6) -Purple GNPs (200 mg/kg/day each, IP) (n=6)	- <i>Cyanothece</i> sp. was able to produce GNPs -Treatment with the combination extract+blue-GNPs and purple-GNPs improved the ISO induced escalation of the diagnostic cardiac indicator enzymes (CK, CK-MB, cTnT, and LDH), in comparison with ISO-control rats -Treatment with extract+blue-GNPs or purple-GNPs prohibited the ISO-induced deterioration in SBP, DBP, and MBP -The increase in HR was also attenuated in extract+GNP rats, compared to the ISO control rats -Treatment with extract+GNPs showed a reverse in ECG-induced alterations presented in the ISO treated rats -Treatments with extract+GNPs increased the activity of the GSH and SOD, which were decreased in rats injected with ISO rats
		Male Sprague-Dawley rats: acclimatation with ISO (n=24) to induce infarction (100 mg/kg)	-Extract (200 mg/kg/day, IP) (n=6) -Mixture of extract+blue-GNP (200 mg/kg/day each, IP) (n=6) -Mixture of extract+purple GNP (200 mg/kg/day each, IP) (n=6)	-Injection with normal saline (IP) (n=6)	
Stapleton et al. (25)	To investigate a photosynthetic oxygen delivery system that rescues the myocardium following acute ischemia	<i>In vitro</i> : neonatal rats CM (hypoxic chamber) (n=12)	CM+20x10 <sup>3</sup> <i>S. elongatus</i> -HMP (n=3)	-CM+PBS (control) (n=3) -CM+1x10 <sup>6</sup> <i>S. elongatus</i> (therapeutic control) (n=3) -CM+20x10 <sup>3</sup> HMP (vehicle control) (n=3)	-HMP improve the viability of <i>S. elongatus</i> during the injection process -Treatment with <i>S. elongatus</i> -HMP mitigated cellular apoptosis and improved LV function
		<i>In vivo</i> : male Winstar rats. (cardiac puncture of the left ventricle) (n=45) -Acute ischemia reperfusion (n=20) -Chronic ischemia reperfusion (n=20)	Injection of 20x10 <sup>3</sup> <i>S. elongatus</i> -HMP (n=5) (AIR and CIR)	-Non-injected <i>S. elongatus</i> (2x, n=5) -Injections (AIR and CIR): PBS (2x, n=5) 1x10 <sup>6</sup> PBS+ <i>S. elongatus</i> (n=5) PBS+20x10 <sup>3</sup> HMP (2x, n=5)	

AIR, Acute Ischemia/Reperfusion; CIR, Chronic Ischemia/Reperfusion; CK, Creatine Kinase; CM, Cardiomyocytes; cTnT, Cardiac Troponin; DBP, Diastolic Arterial Blood Pressure; ECG, Electrocardiogram; GNP, Gold Nanoparticles; GRx, Glutaredoxin; HMOX1, Heme Oxygenase-1; HMP, Hydrogel Microparticles; HR, Heart Rate; IP, Intraperitoneal; IR, Ischemia/Reperfusion; ISO, isoproterenol; LAD, Left anterior descending; LDH, Lactate Dehydrogenase; LV, Left Ventricle; LVDP, Left Ventricular Developed Pressure; MBP, Mean Arterial Blood Pressure; MPO, Myeloperoxidase; MI, myocardial infarction; NIR, Near-Infrared Irradiation; PBS, Phosphate Buffered Saline; PC, Phycocyanin; PCB, Phycocyanobilin; RPP, Rate Pressure Product; SBP, Systolic Arterial Blood Pressure; SOD, superoxide dismutase; SP, Spirulina.

### Study characteristics

For *in vitro* studies, human endothelial EA.hy926 cells, cardiac HgC2 cells, CM cultures and hearts isolated from rats were used. To induce damage, the tissues were kept in hypoxia and/or glucose deprivation or treated with  $H_2O_2$ . For *in vivo* studies, male Sprague-Dawley and Wistar rats were used, as well as ApoE-deficient female mice. To induce ischemia-damage, the methods used were abdominal aorta clamping (ischemia) and releasing (reperfusion), Left anterior descending (LAD) permanent (ischemia) or temporary (IR) occlusion, isoproterenol (ISO) injection and cardiac puncture of the left ventricle.

### Intervention

For *in vitro* studies, Spirulina (*A. platensis*) or *S. elongatus* were used in co-cultures with cell lineages under dark and light conditions. Crude cyanobacterial extracts, phycocyanobilin (PCB), phycocyanin (PC), and nanoparticles/hydrogels combined with *S. elongatus* were also applied. For *in vivo* studies, Spirulina ingestion, applications of cyanobacterial extracts (IP), direct injection of *S. elongatus* into cardiac tissues, and injection of nanoparticles/hydrogels combined with *S. elongatus* (under dark and light conditions) were utilized.

### Main Outcomes

The main outcomes evaluated were antioxidant effects of the treatments, effects on blood biochemical markers, oxygenation capacity of Cyanobacteria, and cardiac recovery after ischemia and IR.

### Synthesis of the results

In general, two types of interventions for treatment of ischemia and IR in cardiac tissue were found: (1) treatments using cyanobacterial extracts and (2) injection of living Cyanobacteria in the damaged tissues. The treatments using cyanobacterial extracts are based in their antioxidant activity, and the studies regarding to these methods focus mainly in the extracts of the species *A. platensis*, commercially known as "Spirulina". The treat-

ments regarding to the direct injection of living Cyanobacteria in the cardiac tissue are based in the high capacity of these organisms to release oxygen during photosynthesis. *S. elongatus* is the Cyanobacteria species most commonly utilized in injections, either delivered independently or carried by hydrogels or nanoparticles. Unlike other gram-negative bacteria, the structurally unique lipopolysaccharide present on the surface of this species does not provoke important inflammatory response (13). This characteristic renders *S. elongatus* a promising candidate for *in vivo* applications as an oxygen source in MI (14). Moreover, the coccoid Cyanobacteria *S. elongatus* is easily cultivated and there's no report of toxin production by strains of this species (13), which makes it suitable for medical treatments. There is also one study testing injections with the coccoid Cyanobacteria genus *Cyanothece* Komárek, which is also nontoxic (15), and, there is one study testing *Lyngbya majuscula* Gomont applications (16), a filamentous marine Cyanobacteria, which unfortunately can produce toxins (17), and must be studied more carefully for medical treatments.

### Discussion

#### *Antioxidant Potential of Cyanobacteria for Cardioprotection against Ischemic Injury*

PCB is a specific type of PC protein, which serves as a light-harvesting pigment found in Cyanobacteria, including the commercially significant Spirulina (9). PCs have strong antioxidant and anti-inflammatory effects and can be used in the treatment of a variety of diseases by scavenging reactive oxygen species, such as cataracts, nonalcoholic fatty liver, and degenerative diseases (10). PCB presents structural similarities with human bile pigment bilirubin, which is a potent endogenous antioxidant. Many experimental and clinical studies show that bilirubin have significant protective effects against oxidative stress-related diseases (18). Moreover, many clinical studies have identified a negative correlation between serum bilirubin levels and the incidence of cardiovascular diseases. The production of bilirubin in the human body is regulated by heme oxygenase (HMOX), and specially the isoform Heme Oxygenase-1 (HMOX1)

is highly inducible, and its expression is upregulated in response to various pro-oxidant stimuli. Evidence indicate that HMOX1 expression in the vasculature exerts protective effects against atherosclerotic processes, primarily through its antioxidant, anti-inflammatory, anti-apoptotic, and potentially immunomodulatory properties (19).

Strasky et al. (20) tested the *in vivo* and *in vitro* effects of treatments with Spirulina and PCB on the regulation of HMOX1. The findings show that both Spirulina and PCB upregulate the expression of HMOX1. This enzyme is responsible for the cleavage of the heme (hemoglobin precursor) in biliverdin. The biliverdin is rapidly reduced to bilirubin, which is an important antioxidant. These findings suggest that the supplementation with Spirulina and PCB can be used to prevent and treat atherosclerotic diseases, considering the antioxidant activity of the upregulation of HMOX1 enzyme. Another important study concerning the applications of PC and Spirulina against IR injuries is Khan et al. (21). This study aimed to investigate the cardioprotective role of PC and Spirulina against IR injury and the underlying signaling mechanisms involved. Rat hearts were isolated and subjected to experiments as outlined in Table 1. The results demonstrate that following IR, hearts infused with PC or SP exhibited higher percent recovery of left ventricular development pressure and rate pressure product. These findings serve as an indirect index of myocardial oxygen consumption and indicates the positive effects of PC and Spirulina on cardiac function.

Biochemical markers were also assessed, revealing that levels of Lactate Dehydrogenase (LDH) in the effluent from control hearts increased during reperfusion, while LDH activity decreased in coronary effluent from hearts treated with PC and Spirulina. LDH is an important molecular marker of tissue injury and is released from cells following an ischemic event. Similarly, Creatine Kinase (CK) activity in coronary effluent increased after IR in control, but hearts treated with PC or Spirulina showed reduced IR-induced increase in CK activity in coronary effluent. Both LDH and CK are molecular markers for tissue injuries. Mo-

reover, PC and Spirulina treatments reduced the infarct size, attenuated generation of free radicals during reperfusion and reduced the number of apoptotic cell (21).

Another study reporting the effects of Spirulina as a cardiovascular protector is Sari et al. (2). This study investigated the impact of Spirulina on multiorgan damage induced by IR using a rat model. The authors employed biochemical and histopathological approaches to evaluate the outcomes. The results of the study demonstrated increased total antioxidant capacity and superoxide dismutase (SOD) levels, along with decreased total oxidant status, oxidative stress index, and myeloperoxidase levels. SOD is an essential enzyme that catalyzes the dismutation of superoxide into oxygen and hydrogen peroxide, thereby mitigating oxidative stress and potentially inhibiting the formation of atherosclerotic plaques. Myeloperoxidase, produced in neutrophils, plays a role in inflammatory processes and exhibits oxidant antibacterial activity (22).

The histopathological findings reveal that Spirulina reduced IR-related apoptosis and tissue damage such as cell swelling (2). Cell swelling can culminate in destruction of membranes and is a consequence of the depletion of ATP in ischemia, which causes sodium/potassium pump failure and exacerbated water uptake into the cells (1). Also, focal necrosis, and neutrophil infiltration in the interstitial area and between CM were reduced in treatment, after IR injury (2).

C-phycocyanin was also investigated by Gao et al (6). The authors explored the effects of this pigment on regulating mitochondrial dynamics in IR CM through *in vitro* experiments. Keeping a dynamic equilibrium between mitochondrial fusion and fission is crucial for determining their morphology, quantity, subcellular distribution, and overall functionality. However, ischemic and IR events can unbalance these dynamics towards mitochondrial fission, culminating in apoptosis. In this study, the PC treatment effectively restored the imbalanced mitochondrial dynamics induced by IR, by inhibiting mitochondrial fission and promoting fusion, demonstrating that treatment

with PC had a protective effect against cell death following IR. Furthermore, the PC-treated groups exhibited reduced intracellular production of ROS after IR.

### *Injection of Cyanobacteria for Cardioprotection against Ischemic Injury*

Beyond the antioxidant effects and the consequent application of Cyanobacteria in heart ischemia and IR, innovative techniques emerged using these organisms as a source of oxygen supply in these situations. Oxygen therapy is a common approach in ischemia treatment, but high concentrations of oxygen can result in vasoconstriction and increased vascular resistance, exacerbating cell damage. However, Cyanobacteria, particularly *S. elongatus*, have shown the ability to release oxygen in damaged cardiac tissues in a controlled manner, offering a promising alternative (14).

This ability of Cyanobacteria is due to its capacity to perform oxygenic photosynthesis. They have the photosystem II (responsible to release oxygen) and a wide variety of pigments, such as Chlorophylls a, b, d and f, which permits them to absorb infrared (700-750 nm), in addition to the standard photosynthetically active radiation (400-700 nm) (23). Also, it is known that Cyanobacterial therapy is nontoxic and nonpathogenic (24).

Considering that, Cohen et al. (24) performed *in vitro* and *in vivo* tests to evaluate the effects of injection of *S. elongatus* in ischemic cardiovascular tissue. The tests were performed under light and dark conditions to analyze the capacity of the photosynthetic organism to supply the tissues during oxygen depletion. The *in vitro* tests showed that *S. elongatus* did not affect the survival of CM and proved to be photosynthetically active when cocultured with cardiovascular cells under mammalian physiological conditions. Also, demonstrated that CM cocultured with *S. elongatus* in the light presented higher oxygenation than did CM alone. In hypoxia conditions, the group treated with light demonstrated increase in cellular metabolism when compared to the dark group, indicating positive effects of the oxygenation from

the Cyanobacteria.

The *in vivo* studies were carried on ischemia and IR in light/dark conditions. For ischemia treatments, in the light, *S. elongatus*-treated hearts demonstrated a nearly 25-fold increase in oxygenation levels. Moreover, the *S. elongatus* group demonstrated an increase in local temperature from the time of ligation (induced ischemia), indicating enhanced metabolic activity and enhanced ventricular contractility and overall cardiac performance. For IR treatments, serum troponin was significantly reduced in the *S. elongatus*-treated group. The serum troponin is a diagnostic marker for infarction and this result indicates the positive effect of the Cyanobacteria against cell damage. Also, in this group, the end-systolic volume was lower, and the Left Ventricle (LV) ejection fraction was augmented.

*S. elongatus* was also tested by (25). The authors encapsulated *S. elongatus* into alginate hydrogel microparticles (HMP), in order to provide mechanical support to the Cyanobacteria during injection and enhance therapeutic potential by adopting a minimally invasive approach. The *S. elongatus*-HMP were injected in the hearts of rats submitted to acute and chronic ischemia/reperfusion events. Also, tests were made *in vitro*, using neonatal rats CM treated with *S. elongatus*-HMP, in culture plates. Results showed that HMP improve the viability of *S. elongatus* during the injection process and the treatment with *S. elongatus*-HMP mitigated cellular apoptosis and improved left ventricular function.

All of these studies above consider the capacity of Cyanobacteria to produce oxygen in light conditions but ignore the respiration of these organisms in dark conditions. Taking this in account, Liu et al. (14) developed a hydrogel-coated upconversion cyanobacterium nanocapsule (UCCy@Gel) for ischemia prevention and therapy. For that, they modified a *S. elongatus* strain and covered the cells with a methacrylate hydrogel. This nanocapsule is capable to absorb penetrable near-infrared (NIR) photons in deep tissues and emit shorter wavelength photons (upconversion luminescence) that can be used for photosynthesis and oxygenate the

injured tissues. Moreover, the oxygen generated under light irradiation suppressed macrophage polarization to the M1 state, reduced the levels of pro-inflammatory (TNF- and IL-6), and increased the levels of anti-inflammatory cytokines. In the dark, this nanocapsule present protective effects on CM. The hypoxic environment created by the Cyanobacteria respiration upregulates the heat shock protein70 (HSP70), which inhibits the expression of the apoptosis protein cysteinyl aspartate specific proteinase-3 (Caspase-3) and the apoptosis reactions cascade, consequently preventing the loss of CM and cardiovascular function after ischemia.

A different approach was adopted by (16), which explored the potential of the Cyanobacteria *L. majuscula* to produce gold nanoparticles (GNPs) and use them to treat ischemic heart injuries. GNPs are involved in many medical applications and possess a variety of pharmaceutical and pharmacological properties. They can be used delivering drugs in the treatment of cancer, Alzheimer's disease, HIV, hepatitis B, tuberculosis, diabetes, and influenza (16). In this study, the authors induced rat's hearts infarction by the intraperitoneal administration of ISO (Table 1). The results show that the treatment with GNPs alone, and the combination of cyanobacterial extract with GNPs, ameliorated the ISO-induced escalation of the diagnostic cardiac indicator enzymes (CPK, CK-MB, and Cardiac Troponin), in comparison with ISO-control rats. The treatment with extract alone was not efficient, different from the other papers that we reviewed. In this case, the importance of the Cyanobacteria is restricted to the production of the GNPs.

The same results were found for the arterial pressure indices. Treatment with GNPs, and extract+GNPs prevented the ISO-induced decline of the Systolic blood pressure (SBP), diastolic blood pressure (DBP) and mean blood pressure (MBP). Similarly, the increase in heart rate (HR) was also attenuated in GNP and extract+GPNP rats, when compared to the ISO control rats. The Electrocardiogram (ECG)-induced alterations presented in the ISO treated rats were reversed in the treatments with GNPs and extract+GPNP. In this paper,

the cyanobacterial extract alone only presented activity increasing the activity of Glutaredoxin (GRx) and SOD, which were decreased in rats injected with ISO. This antioxidant property agrees with the other articles revised in our paper. Moreover, the treatments with GNP and extract+GPNP presented the same results for these enzymes activities.

The same experimental design was conducted by Younis et al. (15) to test the applicability of the coccoid Cyanobacteria *Cyanothece* sp. Just as *L. majuscula*, *Cyanothece* sp. was able to produce the GNPs. The other results were also very similar as follows: the treatment with the combination extract+blue-GNPs and purple-GNPs improved the ISO induced escalation of diagnostic cardiac indicator enzymes (CPK, CK-MB, Cardiac Troponin, and LDH), in comparison with ISO-control rats. The treatment with extract alone was not efficient for these markers. Considering arterial pressure indices, the treatment with extract+blue-GNPs or purple-GNPs prohibited the ISO-induced deterioration in SBP, DBP, and MBP. Similarly, the increase in HR was also attenuated in extract+GPNP rats compared to the ISO control rats. The Electrocardiogram (ECG)-induced alterations presented in the ISO treated rats were reversed in the treatments with extract+GNPs. Finally, the treatments with extract+GNPs increased the activity of the antioxidant enzymes Glutathione (GSH) and SOD, which were decreased in rats injected with ISO rats, promoting CM protection against IR.

### Conclusion and Limitations for Clinical Uses

In conclusion, the studies discussed in this text highlight the potential benefits of PC and Spirulina in mitigating the effects of IR injury and protecting against oxidative stress-related diseases. PC and Spirulina exhibit strong antioxidant and anti-inflammatory properties, which can scavenge reactive oxygen species and provide therapeutic effects. Additionally, the studies demonstrate that PC and Spirulina treatments improve cardiac function following IR and regulate biochemical markers associated with tissue injury, such as LDH and CK. Furthermore, PC treatment has been found to protect CM from apoptosis.

Considering the injection of Cyanobacteria directly or through a carrier, *S. elongatus* have demonstrated great ability to release oxygen in damaged cardiac tissues in a controlled manner, offering a potential alternative to traditional oxygen therapy. This nontoxic species has shown positive effects on cell survival, oxygenation levels, cellular metabolism, and cardiac function. The association with hydrogels or nanoparticles can enhance the effects of this therapy, by improving the viability of the cyanobacterial cells. This innovative approach is promising, but the long-term therapy and the light application for photosynthesis in these cases are still a great challenge.

Overall, these studies highlight the potential of Cyanobacteria in providing oxygen, enhancing cellular metabolism, improving cardiac function, and protecting against ischemic damage. Further research and development in this field may open up new perspectives for the treatment and prevention of myocardial infarction and other ischemic conditions using Cyanobacteria-based therapies.

The limitations for the clinical employment of Cyanobacteria on cardiomyocytes protection and recovery rely mainly on the injection approach. These methods are still in the early stages of research and require optimization. Currently, there is no established protocol or technology for long-term application. To overcome these limitations, it is necessary to improve the light application on Cyanobacteria and develop strains that can safely reside in the human heart without causing harm to the patient.

## Statement

During the preparation of this work the authors used [ChatGPT /OpenAI] to correct the English language in some parts of the text. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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