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# Antioxidant Effect of *Spirulina (Arthrospira) maxima* on Chronic Inflammation Induced by Freund's Complete Adjuvant in Rats

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ABSTRACT One of the major mechanisms in the pathogenesis of chronic inflammation is the excessive production of reactive oxygen and reactive nitrogen species, and therefore, oxidative stress. *Spirulina* (*Arthrospira*) *maxima* has marked antioxidant activity *in vivo* and *in vitro*, as well as anti-inflammatory activity in certain experimental models, the latter activity being mediated probably by the antioxidant activity of this cyanobacterium. In the present study, chronic inflammation was induced through injection of Freund's complete adjuvant (CFA) in rats treated daily with *Spirulina* (*Arthrospira*) *maxima* for 2 weeks beginning on day 14. Joint diameter, body temperature, and motor capacity were assessed each week. On days 0 and 28, total and differential leukocyte counts and serum oxidative damage were determined, the latter by assessing lipid peroxidation and protein carbonyl content. At the end of the study, oxidative damage to joints was likewise evaluated. Results show that *S. maxima* favors increased mobility, as well as body temperature regulation, and a number of circulating leukocytes, lymphocytes, and monocytes in specimens with CFA-induced chronic inflammation and also protects against oxidative damage in joint tissue as well as serum. In conclusion, the protection afforded by *S. maxima* against development of chronic inflammation is due to its antioxidant activity.

**KEY WORDS:** • ***antioxidant effect*** • ***chronic inflammation*** • ***Freund's complete adjuvant*** • ***oxidative damage*** • **Spirulina (Arthrospira) maxima**

## INTRODUCTION

Inflammation is an immune system reaction that occurs in vascularized tissue and is caused by physical, chemical, or biological agents, its purpose being to find, isolate, and eliminate pathogenic agents and repair the damaged area. Diverse pathologies are closely related to chronic inflammation as a part of their pathogenesis, among others, rheumatoid arthritis (RA).[1,2] This chronic degenerative autoimmune disorder is present in 2–4% of the world's population. In Mexico, it has an incidence of 0.3% (0.1–0.6%), 59% of this population being women and 41% men.[3]

One of the most important mechanisms in the pathogenesis of chronic inflammation is the production of reactive oxygen species (ROS) and reactive nitrogen species by activated macrophages.[4] An increase in the levels of free radicals can compromise the structure and function of various important biomolecules, such as proteins and membrane lipids in joint tissue of RA patients, inducing oxidative stress.[5] Although the exact cause of RA is unknown, articular disorders have been associated with important increases in the production of ROS, including free radicals such as the superoxide anion, nitric oxide, and their derivatives. Hence, drugs that combat the damage produced by an excess of ROS in joint tissue should be quite useful for impeding the development and progression of articular disorders like RA.[6]

At present, anti-inflammatory and especially antiarthritic treatments are aimed at blockage of inflammatory mediators or inhibition of the cells producing them to control their degenerative effect on joints of RA patients.[7–9] However, due to the large number of side effects elicited by these pharmaceuticals, patients have increasingly focused their attention on natural remedies.[10]

*Spirulina*, a practically harmless cyanobacterium, has been demonstrated to have marked antioxidant activity *in vivo* and *in vitro*.[11–13] This is attributed to the synergistic action of its components such as antioxidant pigments, phycobiliproteins, carotenes, vitamins C and E, $\gamma$-linolenic acid, and phenolic compounds.[14,15] The antioxidant effect of *Spirulina* may be responsible for its multiple pharmacological activities, which include antiviral,[16] hypoglycemic,[17] hypolipidemic,[11] anticarcinogenic,[18] antianemic,[19] and anti-inflammatory[20] properties.

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It has been shown that the consumption of *Spirulina* can prevent different pathologies associated with oxidative stress and inflammation. Previous studies have determined its anti-inflammatory activity in mouse models of chronic inflammation and RA, by evaluating $\beta$-glucuronidase in synovial fluid and subplantar edema, as well as diverse enzymes such as aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase, but have not associated this effect with the above-mentioned antioxidant activity of this cyanobacterium.[20,21] Besides, *Spirulina* inhibits the activation of the nuclear transcription factor that is activated by ROS and, thereby, also inhibits the expression of cytokines and enzymes like TNF and iNOS, respectively. In this way, cyanobacteria can counter the onset of inflammatory disorders and attenuate their progression.[22] Indeed, C-phycocyanin, which is the main antioxidant compound of *Spirulina*, has been proposed as the principal anti-inflammatory and antiarthritic agent of cyanobacteria, since it exerts an important anti-inflammatory effect through different mechanisms of action. One such mechanism is ROS scavenging.[23] However, this anti-inflammatory effect has not been associated with the antioxidant activity of the complete cyanobacteria in a model of chronic inflammation. Hence, the objective of the present study was to examine whether or not the anti-inflammatory activity of *Spirulina* is related to its antioxidant properties, using a model of chronic inflammation that resembles RA.

## MATERIALS AND METHODS

### *Specimens*

Female Sprague-Dawley rats (90–100 g body weight) obtained from the BIRMEX research animal resource facility (Laboratorios de Biológicos y Reactivos de México, Mexico DF) were acclimated for 7 days, with 12-h light/12-h dark periods, temperature 25°C ± 2°C, 55–80% humidity, and food (Lab Rodent Chow) and water provided *ad libitum*. Handling of specimens was done according to the policies of the U.S. National Institutes of Health and the official Mexican norm (NOM-062-ZOO-1999) regarding the care and use of laboratory animals.[24,25]

### *Reagents*

All reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA).

### *Procurement and culturing of* Spirulina maxima

*S. maxima* was obtained from the Plant Physiology Laboratory of the Department of Biology (Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, ENCB-IPN) and was subsequently cultured in a photoreactor, adding the Zarrouk culture medium and a micronutrient solution as described by Silveira *et al*.[26]

### *Experimental design*

The chronic inflammation model proposed by Helyes *et al*.[27] was used. Specimens were randomly assigned to one of the six groups ($n = 8$): (1) negative control; (2) Freund's complete adjuvant (CFA) control; (3) phenylbutazone (PB); (4) *S. maxima* (SP); (5) CFA + PB; and (6) CFA + SP. Chronic inflammation was induced by subcutaneous injection of 300 $\mu$L of a 1:1 emulsion of phosphate-buffered saline (PBS) pH 7.3 and CFA (1 mg inactivated *Mycobacterium tuberculosis* per mL malonate) at the base of the tail. Negative control specimens were injected 300 $\mu$L PBS only. After 14 days of CFA injection, daily treatment with PB or SP was initiated intragastrically at doses of 100 and 800 mg/kg, respectively, for 2 weeks. Untreated specimens were administered the vehicle only. Injection of CFA or PBS was taken as day 0 of the study. On days 0, 7, 14, 21, and 28, the joint diameter, body temperature, and motor capacity were evaluated. Total and differential leukocyte counts as well as serum oxidative damage by assessment of lipid peroxidation (LPO) and protein carbonyl content (PCC) were evaluated on days 0 and 28. At the end of the study, specimens were sacrificed and oxidative damage to wrist and ankle joints was determined.

### *Evaluation of joint inflammation and body temperature*

Chronic inflammation in the wrist and ankle joints of each specimen was measured with a digital caliper, and results were expressed as the increase in joint diameter in mm.[21] The rectal temperature was taken and recorded in degrees centigrade.

### *Determination of motor capacity*

Motor capacity was evaluated by the open field test (OFT) during the last darkness period using a 50 × 50 cm arena subdivided into 5 × 5 cm squares. Each specimen was placed in the center of the field and the number of crossings made during 5 min was recorded. The field was wiped clean with 5% acetic acid after each measurement.[28]

### *Total and differential leukocyte counts*

Blood samples were obtained by retro-orbital puncture using EDTA as the anticoagulant, and blood smears were prepared and stained with Wright's procedure for examination in the microscope. The total number of leukocytes was counted, recording also the number of monocytes and lymphocytes.

### *Oxidative damage*

Blood samples were centrifuged at 1,500 × $g$ for 15 min to obtain serum. Specimens were euthanized in an ether-saturated chamber and joint tissue was removed from the wrists and ankles in an ice bath. Tissue samples 500 mg in weight were homogenized in 2 mL PBS at pH 7.3. Then, 1 mL of each (homogenate or serum) sample was centrifuged at 10,500 × $g$ and 4°C for 15 min and the total protein content was determined in the supernatants. LPO and PCC were evaluated in uncentrifuged homogenate or serum samples. The total protein content was determined by Bradford's method[29] and was used to express results.

### *Determination of LPO*

LPO was evaluated by assessing the levels of thiobarbituric acid reactive substances (TBARS). To 500 $\mu$L of homogenate or 100 $\mu$L of serum was added 2 mL TBARS reagent (16% trichloroacetic acid [TCA], 0.5% thiobarbituric acid, and 0.3 N HCl). The samples were incubated at boiling for 15 min, then placed in an ice bath for 10 min, and centrifuged at $1,000 \times g$ and 4°C for 10 min. Absorbance was read at 535 nm. The malondialdehyde (MDA) content was determined using the molar extinction coefficient (MEC) of $1.56 \times 10^5$ $M^{-1}$ $cm^{-1}$ and results were expressed as $\mu$mol or nmol MDA/mg protein/g tissue or mL serum.[30]

### *Protein carbonyl content*

To 300 $\mu$L of homogenate or 100 $\mu$L of serum was added 300 $\mu$L of 20% TCA, and the samples were subsequently processed as described elsewere.[31] Readings were done at 360 nm. PCC was determined using the MEC of 21,000 $M^{-1}$ $cm^{-1}$ and results were expressed as $\mu$mol or nmol reactive carbonyls (C = O)/mg protein/g tissue or mL serum.

### *Statistical analysis*

The results obtained for markers of general toxicity, leukocyte counts, and oxidative damage parameters in serum were analyzed by two-way repeated measures analysis of variance (ANOVA), while oxidative damage results obtained from joint tissue were subjected to ANOVA on ranks ($P < .05$). Differences between means were determined with the Student–Newman–Keuls *post hoc* test. SigmaStat v3.5 was used throughout.

## RESULTS

No significant increase in joint diameter occurred in CFA-immunized specimens compared to the negative control group, which indicates that at the macroscopic level, neither edema nor inflammation was developed (data not shown).

Regarding body temperature, hyperthermia was observed in the group injected with the phlogistic agent only ($P < .05$) compared to the control group. However, in PB- or SP-treated groups, this increase was prevented and normal values were attained (Table 1).

In the OFT conducted to evaluate motor capacity, both untreated and PB-treated CFA-immunized rats showed a significant loss of mobility on days 14 and 28 compared to negative control specimens ($P < .05$). SP-treated CFA-immunized rats had significantly increased mobility on day 28 in comparison to the positive control group, matching even negative control group values (Table 1).

On the other hand, injection of CFA resulted in the development of leukocytosis on day 28 by comparison to the negative control group, while specimens treated with CFA + PB or CFA + SP evidenced a significant reduction of this condition compared to the positive control group, although normal values were not recovered with either treatment. Furthermore, differential staining revealed monocytosis and lymphocytosis in the positive control group on day 28 by comparison with the negative control group. A significant reduction in both conditions compared to the positive control group occurred on day 28 in groups treated with CFA + PB or CFA + SP; however, treatment with the pharmaceutical agent was more effective ($P < .05$) (Table 2).

Regarding oxidative damage, both serum proteins and lipids were affected in all groups in which chronic inflammation was induced ($P < .05$) (Table 3). However, administration of PB or SP prevented LPO totally and

Table 1. Changes in Body Temperature and Motor Capacity in Specimens with Chronic Inflammation Treated with *Spirulina maxima* ($n = 8$)

| *Treatment* | *Day* | *Body temperature (°C)* | *Mobility (crossings/5 min)* |
|---|---|---|---|
| Negative control (PBS) | 0 | 37.12 ± 0.15 | 129.8 ± 3.35 |
| | 7 | 38.1 ± 0.29[•] | 131 ± 21.47 |
| | 14 | 37.64 ± 0.41 | 144.8 ± 11.80 |
| | 21 | 37.4 ± 0.18* | 100.8 ± 12.72[•*▲] |
| | 28 | 37.1 ± 0.37* | 143.6 ± 13.67 |
| Positive control (CFA) | 0 | 37.62 ± 0.18 | 147 ± 5.24 |
| | 7 | 37.88 ± 0.2 | 125.6 ± 10.41 |
| | 14 | 38.42 ± 0.22[a•] | 95.4 ± 4.50[a•*] |
| | 21 | 37.9 ± 0.13 | 88.4 ± 9.65[•*] |
| | 28 | 38.38 ± 0.28[a•] | 100.8 ± 19.58[a•*] |
| PB 100 mg/kg | 0 | 37.68 ± 0.17 | 122 ± 1.41 |
| | 7 | 37.4 ± 0.17 | 126 ± 1.41 |
| | 14 | 37.1 ± 0.18 | 131.2 ± 0.86 |
| | 21 | 36.86 ± 0.30[•] | 123.6 ± 2.20 |
| | 28 | 37.8 ± 0.23[a▲◆] | 117.6 ± 1.69 |
| SP 800 mg/kg | 0 | 37.44 ± 0.08 | 134.4 ± 5.64 |
| | 7 | 37.66 ± 0.18 | 126 ± 2.00 |
| | 14 | 37.3 ± 0.15 | 132.6 ± 1.72 |
| | 21 | 37.28 ± 0.14 | 135.2 ± 2.00[a] |
| | 28 | 37.36 ± 0.23 | 120 ± 2.16 |
| CFA + PB 100 mg/kg | 0 | 36.6 ± 0.14 | 114.6 ± 14.76 |
| | 7 | 37.34 ± 0.09[a•] | 112.6 ± 10.38 |
| | 14 | 37.54 ± 0.08[b•] | 99.4 ± 7.82[a] |
| | 21 | 37.32 ± 0.06[•] | 84 ± 5.03[•*] |
| | 28 | 36.68 ± 0.14[b*▲◆] | 104.6 ± 1.63[a] |
| CFA + SP 800 mg/kg | 0 | 37.1 ± 0.07 | 137 ± 2.36 |
| | 7 | 37.42 ± 0.10 | 120.4 ± 1.93 |
| | 14 | 37.24 ± 0.12[b] | 101.2 ± 3.03[a•] |
| | 21 | 36.56 ± 0.05[abc*▲] | 114.6 ± 2.13[c] |
| | 28 | 36.56 ± 0.06[b*▲] | 124.2 ± 1.98[b] |

Values are the mean ± SE. Two-way repeated measures ANOVA, SNK *post hoc* test ($P \leq .05$).

[a]Versus negative control.

[b]Versus positive control.

[c]Versus CFA + FB.

[•]Versus d0.

*Versus d7.

[▲]Versus d14.

[◆]Versus d21.

PBS, phosphate-buffered saline; CFA, Freund's complete adjuvant; SE, standard error; ANOVA, analysis of variance; SNK, Student–Newman–Keuls.

Table 2. Total Leukocyte Count and Differential (Monocytes and Lymphocytes) Count in Blood of Specimens with Chronic Inflammation Treated with *Spirulina maxima* ($n=8$)

| *Treatment* | *Day* | *Total leukocytes (cells/mm³)* | *Monocytes (cells/mm³)* | *Lymphocytes (cells/mm³)* |
|---|---|---|---|---|
| Negative control (PBS) | 0 | 2510 ± 164.99 | 490.24 ± 95.44 | 14.88 ± 9.42 |
| | 28 | 3258.8 ± 286.93 | 865.20 ± 79.36 | 32.49 ± 9.60 |
| Positive control (CFA) | 0 | 1716 ± 149.85 | 198.2 ± 44.05 | 3.96 ± 3.96 |
| | 28 | 10030.8 ± 303.58[a•] | 4189.60 ± 137.47[a•] | 3470.04 ± 172.45[a•] |
| PB 100 mg/kg | 0 | 1399 ± 160.53 | 227.02 ± 13.28 | 2.78 ± 2.78 |
| | 28 | 1211 ± 172.23[a] | 668.23 ± 8.77 | 7.3 ± 2.9 |
| SP 800 mg/kg | 0 | 2640 ± 188.40 | 553.76 ± 63.88 | 4.64 ± 4.64 |
| | 28 | 3811.6 ± 245.63 | 946.06 ± 81.06 | 16.53 ± 10.13 |
| CFA + PB 100 mg/kg | 0 | 2433.6 ± 72.02 | 736.99 ± 38.86 | 83.23 ± 15.31 |
| | 28 | 7960 ± 444.52[ab•] | 1789.2 ± 137.32[ab•] | 398 ± 94.71[ab•] |
| CFA + SP 800 mg/kg | 0 | 2662 ± 144.77 | 1021.52 ± 52.37 | 128.58 ± 15.91 |
| | 28 | 7265 ± 497.21[ab•] | 2935.6 ± 147.66[abc•] | 1115.35 ± 173.45[abc•] |

Values are the mean ± SE. Two-way repeated measures ANOVA, SNK *post hoc* test ($P<.05$).
[a]Versus negative control.
[b]Versus positive control.
[c]Versus CFA + PB.
[•]Versus d0.

protein oxidation partially since, despite the fact that these treatments induced a decrease in the latter biomarker ($P<.05$), normal PCC values were not recovered and a significant difference was nevertheless maintained with respect to the control group specimens.

Joint tissue of CFA-immunized rats showed a significant increase in both PCC and LPO in comparison to negative control specimens. CFA-immunized groups that were subsequently treated with PB or SP evidenced a significant reduction in PCC compared to the positive control group (Table 3). LPO decreased ($P<.05$) in CFA-immunized groups treated with PB or SP compared to the positive control group, and treatment was more effective with the cyanobacterium than with the pharmaceutical.

## DISCUSSION

RA is a chronic autoimmune disorder characterized by inflammation of the joints, in which joint deformation, loss of mobility, and eventually, bone resorption may occur. Its diverse manifestations at the cellular and molecular levels include inflammation of the synovial membrane, increased leukocyte (lymphocyte and macrophage) infiltration into the synovial space, release of lysosomal enzymes, and overexpression of proinflammatory cytokines.[32]

Free radicals act as immunostimulators of the inflammatory process, since they serve as chemoattractors for immunocompetent cells, bringing lymphocytes to the site of damage during the late or chronic stage of the immune

Table 3. Oxidative Stress Biomarkers in Joint Tissue and Serum of Specimens with Chronic Inflammation Treated with *Spirulina maxima* ($n=8$)

| | | *Serum* | | *Joint tissue* | |
|---|---|---|---|---|---|
| *Treatment* | *Day* | *PCC (nmol carbonyl/mL)* | *LPO (nmol MDA/mL)* | *PCC (μmol carbonyl/g)* | *LPO (μmol MDA/g)* |
| Negative control (PBS) | 0 | 202.47 ± 22.49 | 35.14 ± 3.64 | | |
| | 28 | 172.83 ± 25.30 | 25.97 ± 3.17 | 1.09 ± 0.19 | 0.68 ± 0.15 |
| Positive control (CFA) | 0 | 259.70 ± 11.35 | 32.05 ± 0.69 | | |
| | 28 | 706.18 ± 71.52[a•] | 57.97 ± 6.52[a•] | 7.53 ± 0.96[a] | 2.07 ± 0.26[a] |
| PB 100 mg/kg | 0 | 288.55 ± 17.60 | 21.78 ± 1.69 | | |
| | 28 | 162.78 ± 5[•] | 15.84 ± 0.85 | 1.19 ± 0.03 | 0.48 ± 0.01 |
| SP 800 mg/kg | 0 | 127.66 ± 30.60 | 10.47 ± 1.42 | | |
| | 28 | 124.90 ± 2 | 11.33 ± 1.73 | 0.53 ± 0.05[a] | 0.24 ± 0.004[a] |
| CFA + PB 100 mg/kg | 0 | 297.71 ± 11.43 | 24.69 ± 13.32 | | |
| | 28 | 392.61 ± 41.66[ab•] | 19.33 ± 0.94[b] | 2.63 ± 0.25[ab] | 2.86 ± 1.07[a] |
| CFA + SP 800 mg/kg | 0 | 384.03 ± 53.95 | 29.39 ± 4.68 | | |
| | 28 | 274.84 ± 11.91[abc•] | 34.75 ± 3.37[b] | 2.39 ± 0.16[ab] | 0.5 ± 0.06[bc] |

Values are the mean ± SE. Two-way repeated measures ANOVA (serum) and ANOVA on ranks (joint tissue), SNK *post hoc* test ($P<.05$).
[a]Versus negative control.
[b]Versus positive control.
[c]Versus CFA + PB.
[•]Versus d0.
PCC, protein carbonyl content; LPO, lipid peroxidation.

response. Additionally, free radicals stimulate immunocompetent cells to generate higher levels of proinflammatory mediators, because oxidative stress induces the transcription of numerous genes that code for the synthesis of proinflammatory mediators or for the enzymes that generate such mediators.[33] An increase in ROS leads to lysis of the membranes of the cells adjacent to the site of damage through oxidative processes like lipoperoxidation, the latter of which generates not only lipidic radicals but also the release of arachidonic acid. In this way, high levels of ROS stimulate the production of phospholipase 2A and later inducible cyclooxygenase (COX-2), triggering the generation of more inflammatory mediators, including prostaglandins (PEG2) and leukotrienes, thus exacerbating chronic inflammation.[34]

The most commonly used animal experimental models make use of CFA, which induces edematous inflammation of the joints, leukocytic infiltration, increased proinflammatory cytokines, hyperthermia, loss of body mass, reduced mobility, hyperalgesia, and oxidative stress.[35]

Regarding body temperature, CFA-immunized rats showed hyperthermia both during and at the end of the study. This is in agreement with studies, which have demonstrated that the presence of cell components such as cell wall lipopolysaccharides of certain bacteria (*e.g.*, *M. tuberculosis*) induces overproduction by macrophages of IL-1$\beta$, IL-6, and TNF-$\alpha$, which act as pyrogens at the affected site or at a systemic level.[36,37] Specimens injected with CFA and treated with SP showed reduced body temperature in comparison to the positive control group. This is probably due to the inhibition of a temperature-regulating cytokine[38] or to the arachidonic acid pathway being disabled by suppression of the enzyme COX-2 and, therefore, impairment also of prostaglandin synthesis,[23] as a result of nutritional components and algal pigments such as phycocyanin and $\gamma$-linolenic acid.

Similarly, specimens with chronic inflammation showed reduced motor capacity beginning on day 7 and up to the end of the study. This is consistent with a study which, using a 42-day model of CFA-induced monoarthritis, found that the motor activity decreased on days 3–42, mostly as a result of allodynia, while treatment with various *Curcuma zedoaria* extracts induced increased mobility from day 14 to the end of the experiment.[39] In the present study, specimens with chronic inflammation that were treated with SP showed increased mobility at the end of the experiment, perhaps due to inhibition of the allodynia induced in chronic inflammation conditions.[40] The potential mechanism of pain attenuation may be inhibition of proinflammatory cytokines such as IL-1$\beta$ and TNF-$\alpha$[41] by components present in the cyanobacterium such as long-chain unsaturated fatty acids and the pigments that make up the phycobilisome.[42]

Macrophage activation is a major characteristic of the inflammatory process. These cells release hydrolytic enzymes and ROS,[43] the excessive production of which gives rise to chronic disorders such as RA since the constant action of these species—particularly $O_2^{\bullet -}$—elicits wear in the connective tissue of body joints.[5]

The model of CFA-induced inflammation is mediated mainly by neutrophils in the acute phase and T lymphocytes in the chronic stage, and in both cases, monocytes/macrophages have an active role. In the present study, untreated CFA-immunized specimens developed leukocytosis on day 28, especially of the monocytic and lymphocytic series. These results are consistent with a study that found lymphocyte values below those of the control group in the acute phase but above them on days 25 and 28, events that are characteristic of chronic inflammation.[44] On the other hand, treatment with SP decreased the total leukocyte count, particularly with regard to monocytes and lymphocytes. This reduction is indicative of the fact that chemoattraction and cellular differentiation processes were modified by the alga during the development of CFA-induced inflammation, perhaps due to the suppression of proinflammatory cytokines such as TNF-$\alpha$, IL-1$\beta$, and IL-6,[23,45,46] these being the major signaling agents of the immune process since they act as chemoattractants for macrophages, aiding leukocytic infiltration of the affected site[23,47] and also favoring T-lymphocyte differentiation and maturation.[47,48] This has been confirmed by a study which shows that, when the IL-7 receptor is suppressed, there is inhibition of joint inflammation and cartilage destruction combined with reduced chemoattraction of T lymphocytes into the synovial space, and therefore, a decrease in the levels of cytokines induced by this type of cell.[49]

The anti-inflammatory activity of *Spirulina* is thought to be due, in large part, to a complex of phycobiliproteins known as the phycobilisome and made up of erythrophycocyanin, allophycocyanin, and C-phycocyanin. To the latter are attributed multiple pharmacological activities associated with free radical inhibition. A carrageenan-induced hyperalgesia model has revealed that C-phycocyanin by itself has anti-inflammatory activity since it reduced TNF-$\alpha$ levels and myeloperoxidase activity, selectively inhibiting key enzymes of the acute inflammatory process such as COX-2 and iNOS.[38]

In a chronic inflammatory process such as RA, the antioxidant defense system of the body is compromised. There is a decrease in reduced glutathione (GSH) levels as well as in the activity of antioxidant enzymes—superoxide dismutase (SOD), catalase, and glutathione peroxidase—and therefore increased LPO, suggesting increased oxidative damage induced at the joint level and detected at the system level.[50–52]

In the present study, high PCC and LPO values were recorded in both tissue and serum of CFA-immunized specimens. The oxidative damage detected in serum is indicative of the fact that the inflammatory process has entered the chronic stage, in which the effect is no longer local but rather systemic and exacerbated. Regarding joint tissue, both PCC and LPO increased in CFA-immunized specimens, suggesting marked oxidative damage. These results are consistent with those obtained in a CFA-induced monoarthritis model, in which LPO was observed to increase in arthritic specimens and the body was no longer able to offset the damage with the help of endogenous antioxidants.[51]

In this sense, in chronic inflammatory conditions, reduction in SOD levels and activity has been confirmed, since high TNF-$\alpha$ levels inhibit the expression of two major isoforms of this enzyme (cytoplasmic SOD1 and extracellular SOD3) in both rodents and humans,[6] which may explain the inability of organisms to offset oxidative damage. On the other hand, a significant reduction in PCC and LPO occurred in both serum and joint tissue of SP-treated specimens, probably due to increased antioxidant enzyme activity[53] and the action of algal components. The latter include unsaturated fatty acids such as $\gamma$-linolenic acid,[54–57] cysteine,[45] a precursor of GSH, and C-phycocyanin, which is able to inhibit OH$^\bullet$ and alkoxyl radicals and decreases LPO of the liver microsomal fraction.[23] Besides the well-known activity of *Spirulina* as well as phycocyanin as direct scavengers of free radicals and ROS, it has been demonstrated that phycocyanin and phycocyanobilin (both being biliproteins contained in cyanobacteria) also act to regulate enzymatic systems of immunocompetent cells. One such enzymatic system is NADPH oxidase, which generates both free radicals and ROS during inflammation.[58,59]

In conclusion, the protection afforded by *S. maxima* against the development of chronic inflammation is due to its antioxidant activity.

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## AUTHOR DISCLOSURE STATEMENT

No competing financial interests exist.

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