

Antioxidant Research

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Spirulina fusiformis provides protection against mercuric chloride induced oxidative stress in Swiss albino mice.

[Sharma MK](#), [Sharma A](#), [Kumar A](#), [Kumar M](#).

Department of Zoology, RLS Government (PG) College, Kaladera, Jaipur 303801, India. mkshrma@hotmail.com

Oxidative stress induced by mercuric chloride (5 mg/kg body weight i.p.) in mice substantially increases the lipid peroxidation level along with corresponding decrease in the reduced glutathione and various antioxidant enzymes in liver and increase in serum transaminases activity. Supplementation of Spirulina (800 mg/kg body weight orally, in olive oil, along with mercuric chloride) for 40 days resulted in decreased LPO level, serum glutamate oxaloacetate and serum glutamate pyruvate transaminase activity along with increase in liver GSH level. The activities of antioxidants enzymes superoxide dismutase, catalase and glutathione-S-transferase were also concomitantly restored to near normal level by Spirulina supplementation to mercuric chloride intoxicated mice. The results clearly demonstrate that Spirulina treatment augments the antioxidants defense mechanism in mercuric chloride induced toxicity and provides evidence that it may have a therapeutic role in free radical mediated diseases.

Publication Types:

- Research Support, Non-U.S. Gov't

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In vitro evaluation of protective effects of ascorbic acid and water extract of Spirulina plantesis (blue green algae) on 5-fluorouracil-induced lipid peroxidation.

[Ray S](#), [Roy K](#), [Sengupta C](#).

Division of Pharmaceutical Chemistry, Himalayan Pharmacy Institute, Majhitar, Rangpo, East Sikkim, 737 136, India. supratimray_in@yahoo.co.in

Considering drug-induced lipid peroxidation as a possible mediator of drug-induced toxicity and exploiting the free radical scavenging action of antioxidants, the present study was designed to evaluate the protective effects of ascorbic acid (AA) and water extract of Spirulina plantesis (SP) to minimize 5-fluorouracil (5-FU)-induced lipid peroxidation. The study has been performed in vitro using goat liver as an experimental model. This evaluation was done by measuring the malondialdehyde (MDA), reduced glutathione (GSH), 4-hydroxy-2-nonenal (4-HNE) and nitric oxide (NO) content of the tissue as markers of lipid peroxidation. The results suggest that ascorbic acid and water extract of Spirulina plantesis could suppress the 5-FU-induced lipid peroxidation to a significant extent.

Publication Types:

- In Vitro

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Antioxidant potential of selected *Spirulina platensis* preparations.

Dartsch PC.

Dartsch Scientific GmbH, Institut für zellbiologische Testsysteme, Horb am Neckar, Germany. pc.dartsch@dartsch-scientific.com

Recent studies suggest that *Spirulina*, a unicellular blue-green alga, may have a variety of health benefits and therapeutic properties and is also capable of acting as an antioxidant and antiinflammatory agent. In this study, a cell-free and a cell-based test assay were used to examine the antioxidant and antiinflammatory properties of four selected *Spirulina platensis* preparations: (1) Biospirulina, (2) SpiruComplex, a preparation with naturally bound selenium, chromium and zinc, (3) SpiruZink, a preparation with naturally bound zinc, (4) Zinkspirulina + Acerola, a preparation with naturally bound zinc and acerola powder. The cell-free test assay used potassium superoxide as a donor for superoxide radicals, whereas the cell-based test assay used the formation of intracellular superoxide radicals of functional neutrophils upon stimulation by phorbol-12-myristate-13-acetate as a model to investigate the potential of *Spirulina* preparations to inactivate superoxide radicals. In accordance with the recommended daily dosage, test concentrations ranging from 50 to 1000 microg/mL were chosen. The results showed a dose-dependent inactivation of free superoxide radicals (antioxidant effect) as well as an antiinflammatory effect characterized by a dose-dependent reduction of the metabolic activity of functional neutrophils and a dose-dependent inactivation of superoxide radicals generated during an oxidative burst. The results demonstrate that the tested *Spirulina* preparations have a high antioxidant and antiinflammatory potential. Especially SpiruZink and Zinkspirulina + Acerola might be useful as a supportive therapeutic approach for reducing oxidative stress and/or the generation of oxygen radicals in the course of inflammatory processes.

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A potent anti-oxidant property: fluorescent recombinant alpha-phycoyanin of Spirulina.

[Guan XY](#), [Zhang WJ](#), [Zhang XW](#), [Li YX](#), [Wang JF](#), [Lin HZ](#), [Tang XX](#), [Qin S](#)

Ocean University of China, Qingdao, China.

AIMS: To express and product a fluorescent antioxidant holo-alpha-phycoyanin (PC) of *Spirulina platensis* (Sp) with His-tag (rHHPC; recombinant holo-alpha-phycoyanin of *Spirulina platensis* with His-tag) in 5-l bench scale. **METHODS AND RESULTS:** A vector harbouring two cassettes was constructed: *cpcA* along with *cpcE-cpcF* in one cassette; *ho1-pcyA* in the other cassette. Lyases CpcE/F of *Synechocystis* sp. PCC6803 (S6) could catalyse the 82 site Cys in apo-alpha-PC of Sp linking with bilin chromophores, and rHHPC was biosynthesized in *Escherichia coli* BL21. The constant feeding mode was adopted, and transformant reached the biomass of rHHPC up to 0.55 g l⁻¹ broth in 5-litre bench scale. rHHPC was purified by Ni(2+) affinity column conveniently. The absorbance and the fluorescence emission spectra of rHHPC had lambda(max) at 621 and 650 nm, respectively. The IC(50) values of rHHPC were 277.5 +/- 25.8 microg ml⁻¹ against hydroxyl radicals and 20.8 +/- 2.2 microg ml⁻¹ against peroxy radicals. **CONCLUSIONS:** Combinational biosynthesis of rHHPC was feasible, and the constant feeding mode was adopted to produce good yields of rHHPC. Fluorescent rHHPC with several unique qualitative and quantitative features was effective on scavenging hydroxyl and peroxy radicals. **SIGNIFICANCE AND IMPACT OF THE STUDY:** A potent antioxidant rHHPC was co-expressed, produced and characterized for nutritional and pharmacological values, which would help to develop phycobiliproteins' applications in their fluorescent and biological activities.

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Antioxidant potential of C-phycoyanin isolated from cyanobacterial species *Lyngbya*, *Phormidium* and *Spirulina* spp.

[Patel A](#), [Mishra S](#), [Ghosh PK](#).

Central Salt and Marine Chemicals Research Institute, GB Marg, Bhavnagar, India.

The antioxidant activity of C-Phycocyanin (C-PC) isolated from three cyanobacterial species *Lyngbya* (marine), *Phormidium* (marine) and *Spirulina* (fresh water) was studied in vitro. The results demonstrate that C-PCs from *Lyngbya*, *Phormidium* and *Spirulina* spp. are able to scavenge peroxy radicals (determined by crocin bleaching assay) with relative rate constant ratio of 3.13, 1.89 and 1.8, respectively. C-PCs also scavenge hydroxyl radicals (determined by deoxyribose degradation assay) with second order rate constant values of 7.87×10^{10} , 9.58×10^{10} and 6.42×10^{10} , respectively. Interestingly, *Lyngbya* C-PC is found to be an effective inhibitor of peroxy radicals (IC₅₀ 6.63 microM), as compared to *Spirulina* (IC₅₀ 12.15 microM) and *Phormidium* C-PC (IC₅₀ 12.74 microM) and is close to uric acid (IC₅₀ 2.15 microM). Further, the studies suggest that the covalently-linked tetrapyrrole chromophore phycocyanobilin is involved in the radical scavenging activity of C-PC. The electron spin resonance (ESR) spectra of C-PCs indicate the presence of free radical active sites, which may play an important role in its radical scavenging property. This is the first report on the ESR activity of native C-PCs without perturbations that can cause radical formation.

Publication Types:

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Effect of spirulina maxima on the haloperidol induced tardive dyskinesia and oxidative stress in rats.

[Thaakur SR, Jyothi B.](#)

Department of Pharmacology, School of Pharmaceutical Sciences, Sri Padmavathi Mahila Viswa Vidyalayam, Andhra Pradesh, India. drsanthrani@gmail.com

Haloperidol is a widely used neuroleptic drug for the treatment of acute and chronic psychosis. The use of haloperidol is limited by extrapyramidal movement disorders such as Parkinsonism, akathisia, dystonia, and tardive dyskinesia (TD). Treatment with haloperidol increases oxyradicals which are implicated in TD. Spirulina is widely used as nutritional supplement rich in proteins and antioxidants. The present study is proposed to study the effect of spirulina on haloperidol induced TD and oxidative stress by studying TD, various enzymatic and nonenzymatic antioxidants and lipid peroxidation. Haloperidol 1 mg/kg/i.p was used to induce vacuous chewing movements in rats. Spirulina maxima suspended in 1% between 80 at a dose of 45, 90 and 180 mg/kg were administered by gavage along with haloperidol from 21st day to 49th day of treatment. Spirulina supplementation at a dose of 180 mg/kg significantly improved enzymatic and nonenzymatic antioxidants and decreased the tardive dyskinesia induced by haloperidol. In conclusion, the results of present investigation suggest that spirulina decreases haloperidol induced oxidative stress and TD by many mechanisms as it is cocktail of antioxidants. On chronic use it may inhibit haloperidol induced reduced expression of DNA thereby increases the expression of enzymatic and nonenzymatic antioxidants and protects against oxidative stress induced neurodegeneration and TD.

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Pressurized liquid extracts from *Spirulina platensis* microalga. Determination of their antioxidant activity and preliminary analysis by micellar electrokinetic chromatography.

[Herrero M](#), [Ibáñez E](#), [Cifuentes A](#), [Señoráns J](#).

Instituto de Fermentaciones Industriales (CSIC), Juan de la Cierva 3, 28006 Madrid, Spain.

In this work, different extracts from the microalga *Spirulina platensis* are obtained using pressurized liquid extraction (PLE) and four different solvents (hexane, light petroleum, ethanol and water). Different extraction temperatures (115 and 170 degrees C) were tested using extraction times ranging from 9 to 15 min. The antioxidant activity of the different extracts is determined by means of an in vitro assay using a free radical method. Moreover, a new and fast method is developed using micellar electrokinetic chromatography with diode array detection (MEKC-DAD) to provide a preliminary analysis on the composition of the extracts. This combined application (i.e., in vitro assays plus MEKC-DAD) allowed the fast characterization of the extracts based on their antioxidant activity and the UV-vis spectra of the different compounds found in the extracts. To our knowledge, this work shows for the first time the great possibilities of the combined use of PLE-in vitro assay-MEKC-DAD to investigate natural sources of antioxidants.

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Antioxidant and antiproliferative activities of Spirulina and Chlorella water extracts.

[Wu LC](#), [Ho JA](#), [Shieh MC](#), [Lu IW](#).

Department of Applied Chemistry, National Chi-Nan University, Puli, Nantou, Taiwan. lw25@ncnu.edu.tw

Liver fibrosis is a chronic liver disease that will further develop to cirrhosis if severe damage continues to form. A potential treatment for liver fibrosis is to inhibit activated hepatic stellate cell (HSC) proliferation and, subsequently, to induce HSC apoptosis. It has been reported that antioxidants are able to inhibit the proliferation of HSCs. In this study, the aqueous extract of spirulina was chosen as the source of antioxidant to investigate the inhibitory effect on the proliferation of HSC. The growth inhibitory effects of aqueous spirulina and chlorella extract on human liver cancer cells, HepG2, were also studied and compared in pairs. Results indicated that the total phenol content of spirulina was almost five times greater than that of chlorella (6.86 +/- 0.58 vs 1.44 +/- 0.04 mg tannic acid equivalent/g of algae powder, respectively). The antioxidant activity of spirulina determined by the ABTS*+ method was higher than chlorella (EC50: 72.44 +/- 0.24 micromol of trolox equivalent/g of spirulina extract vs 56.09 +/- 1.99 micromol of trolox equivalent/g of chlorella extract). Results of DPPH* assay also showed a similar trend as the ABTS*+ assay (EC50: 19.39 +/- 0.65 micromol of ascorbic acid equivalent/g of spirulina extract vs 14.04 +/- 1.06 micromol of ascorbic acid equivalent/g of chlorella extract). The aqueous extracts of these two algae both showed antiproliferative effects on HSC and HepG2, but spirulina was a stronger inhibitor than chlorella. Annexin-V staining showed that aqueous extract of spirulina induced apoptosis of HSC after 12 h of treatment. In addition, the aqueous extract of spirulina triggered a cell cycle arrest of HSC at the G2/M phase.

Publication Types:

- Comparative Study
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Characterization via liquid chromatography coupled to diode array detector and tandem mass spectrometry of supercritical fluid antioxidant extracts of *Spirulina platensis* microalga.

[Mendiola JA](#), [Marín FR](#), [Hernández SF](#), [Arredondo BO](#), [Señoráns FJ](#), [Ibañez E](#), [Reglero G](#).

Sección de Ciencias de la Alimentación, Universidad Autónoma de Madrid, Ciudad Universitaria de Cantoblanco, 28049 Madrid, Spain.

Spirulina platensis microalga has been extracted on a pilot scale plant using supercritical fluid extraction (SFE) under various extraction conditions. The extraction yield and the antioxidant activity of the extracts were evaluated in order to select those extracts with both the highest antioxidant capacity and a good extraction yield. These extracts were characterized using LC coupled to diode array detection (DAD) and LC coupled to mass spectrometry (MS) with two different interfaces, atmospheric pressure chemical ionization (APCI) and electrospray (ESI) which allowed us to perform tandem MS by using an ion trap analyzer. The best extraction conditions were as follows: CO₂ with 10% of modifier (ethanol) as extraction solvent, 55 degrees C (extraction temperature) and 220 bar (extraction pressure). Fractionation was achieved by cascade depressurization providing two extracts with different activity and chemical composition. Several compounds have been identified in the extracts, corresponding to different carotenoids previously identified in *Spirulina platensis* microalga along with chlorophyll a and some degradation products. Also, the structure of some phenolic compounds could be tentatively identified. The antioxidant activity of the extracts could be attributed to some of the above mentioned compounds.

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C-phycoyanin: a potent peroxy radical scavenger in vivo and in vitro.

Bhat VB, Madyastha KM.

Department of Organic Chemistry, Indian Institute of Science, Bangalore, 560 012, India.

C-Phycocyanin (from *Spirulina platensis*) effectively inhibited CCl₄-induced lipid peroxidation in rat liver in vivo. Both native and reduced phycocyanin significantly inhibited peroxy radical-induced lipid peroxidation in rat liver microsomes and the inhibition was concentration dependent with an IC₅₀ of 11.35 and 12.7 microM, respectively. The radical scavenging property of phycocyanin was established by studying its reactivity with peroxy and hydroxyl radicals and also by competition kinetics of crocin bleaching. These studies have demonstrated that phycocyanin is a potent peroxy radical scavenger with an IC₅₀ of 5.0 microM and the rate constant ratios obtained for phycocyanin and uric acid (a known peroxy radical scavenger) were 1.54 and 3.5, respectively. These studies clearly suggest that the covalently linked chromophore, phycocyanobilin, is involved in the antioxidant and radical scavenging activity of phycocyanin. Copyright 2000 Academic Press.

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