Indian Journal of Experimental Biology
Vol. 49, October 2011, pp. 739-748

Spirulina platensis protects neurons via suppression of glial activation and peripheral sensitization leading to restoration of motor function in collagen-induced arthritic rats

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Received 11 April 2011; revised 11 July 2011

Spirulina platensis treatment (400 mg kg^{-1} for 25 days) effectively suppressed peripheral sensitization via modulation of glial activation and improved motor coordination and restoration of functional motor activity in collagen-induced arthritic rats. *Spirulina* treatment also resulted in an appreciable reduction of the NF200 accumulation in the spinal cord neurons of arthritic rats. This is indicative of neuroprotective action of *S. platensis* against glutamate excitotoxicity-induced central sensitization produced by the peripheral joint inflammation in the collagen-induced arthritis. The results suggest that effects of *S. platensis* may be due to its counter regulation of spinal glial activation and could be a potential strategy for the treatment of arthritis.

Keywords: Collagen-induced arthritis, Microglia, Neuroprotection, Spinal cord, *Spirulina platensis*

Rheumatoid arthritis (RA), one of the most common inflammatory joint diseases is a major health problem throughout the world with a prevalence of about 0.5-1.0% depending on the geographical distribution[1-3]. Pro-inflammatory cytokines of the inflamed peripheral tissue are known to influence behavioral responses to sensory stimuli leading to the development of hyperalgesia and allodynia[4,5]. In arthritis, the glial cells (both astroglia and microglia) of the spinal cord dorsal horn respond to peripheral inflammatory condition by releasing spinal cytokines which are thought to be involved in central mechanisms underlying the maintenance and exaggeration of pain states termed as noxious stimulation[6-8]. Robust glial activation has been reported in lumbar spinal cord in various rodent models of chronic pain conditions such as spinal cord injury[9], peripheral nerve inflammation and injury[10-12] and chronic opioid treatment[13]. Following inflammation or injury, both microglia and astrocytes become hyperactive and release a variety of algesic molecules that enhance pain transmission, such as proinflammatory cytokines, ATP, nitric oxide, prostaglandins and excitatory amino acids[6, 14-16]. Thus in the pathophysiology of the arthritis it will be of interest to focus on both the joints and neural tissue of the spinal cord.

The collagen-induced arthritis (CIA) model is the most commonly used rat model for RA. Intradermal injection with collagen emulsified in IFA in rats leads to a severe, erosive poly-arthritis developing within 2-3 weeks after immunization followed by a subsequent chronic relapsing phase[17].

Disability associated with RA is largely associated with a decrease in motor activity as a result of pain exacerbated by several inflammatory mediators[16-18]. Decrease in motor activity is also correlated with a decrease in muscle strength and joint destruction in RA patients[16] and can be modulated with anti-inflammatory therapy[19]. FK506, a well known immunosuppressant drug principally used in solid organ transplant recipients, has been reported to improve physical function, pain and joint inflammation in RA patients[20,21] and in experimental injury models[22]. Sasakawa *et al.*[19] reported that FK506 treatment ameliorated spontaneous locomotor activity via improvement of hyperalgesia and muscle strength in collagen-induced arthritic (CIA) rats without suppressing the paw inflammation. However, the study was confined to the behavioral features of arthritis. Very limited information is available on the neuronal and glial changes that occur in the lumbar

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spinal cord as well as in motor coordination and functional motor recovery following CIA. Thus the present study is an attempt to correlate the decreased motor coordination and motor function following CIA with neuronal and glial changes appearing in the spinal cord and to study if, Spirulina could ameliorate these arthritic changes and improve the functional abilities in CIA animals.

Spirulina platensis, a cyanobacterium is used in many countries as a nutritional supplement being a rich source of proteins, vitamins, essential amino acids, minerals, essential fatty acids such as γ-linolenic acid and sulfolipids as well as some more vital elements like calcium, iron, zinc, magnesium, manganese and selenium[23,24]. Moreover, *S. platensis* also contain ω-6 PUFA, vitamin B12, tocopherols and some phytopigments such as natural mixed carotene and xanthophyll, which together with phycocyanin, seem to make it a good antioxidant[25-27].

Early interest in *S. platensis* focused mainly on its potential as a source of protein and vitamins, but recently more attention has been paid to study its therapeutic potential. Oral administration of phycocyanin or Spirulina exerts potent and versatile anti-inflammatory effects in rodents[28-34]. *S. platensis* has also been found to exhibit antiviral[35], anti-platelet[36], anti-cardiotoxic[37], hypocholesterolemic[38], anti-nephrotoxic[32], anti-hepatotoxic[33], anti-acute allergic rhinitis[39] and anti-arthritic effects[40]. Recently *S. platensis* has also been reported to exert chemopreventive effects against dibutyl nitrosamine-induced rat liver cytotoxicity and carcinogenesis, indicating its potential use in chemoprevention of cancer[24].

Arthritic pain is the most commonly reported and disabiliting condition associated with RA. Non-steroidal anti-inflammatory drugs (NSAIDs) used to combat the situations may cause unwanted and dangerous side-effects and other traditional disease modifying anti-rheumatic drugs (DMARD's) such as methotrexate are also usually ineffective[21,41] or result in side effects[42]. Thus effective and safe therapeutic strategy in arthritis is currently most needed. To the best of our knowledge, the neuroprotective effect of *Spirulina platensis* in spinal cord region is being reported for the first time in collagen induced arthritic rats. Only sporadic information is available on the glial changes that occur in the spinal cord[9, 12] as well as motor coordination and functional motor recovery following collagen-induced arthritis. Thus by using specific antibodies and immunocytochemical techniques both the glial activation and neurodegeneration has been studied. Keeping in view the nutritive and therapeutic properties of *S. platensis*, present investigation was thus undertaken to assess whether the *S. platensis* could antagonize activation of glial cells in the spinal cord, delay or attenuate neuroinflammation and neurodegeneration in experimental arthritic rats.

Materials and Methods

Mass cultivation and biomass preparation of S. platensis—*Spirulina platensis* was grown in Zarrouk's medium and the exponentially growing cells were harvested by filtration through screen printing filter of pore size 305 nm (1,400 pore/cm^2). The biomass was dried at 50°C, collected, weighed and used to feed the experimental rats[40].

Experimental model—Female Wistar rats, 8-10 week of age were used in the present study. The choice of sex of the animals, i.e., females, was based on the findings of Holmdahl[43] that autoimmune arthritis is mediated by sex hormones and is associated with a female preponderance for development of arthritis. In addition female rats are more susceptible to experimentally-induced arthritis as compared to the males[43]. The experimental animals were maintained in pathogen free conditions in the animal house on 12 h light/dark cycle under a constant temperature of 25°±1°C and had free access to food and water. Rats were subdivided into following five groups of 3 each: (i) Normal rats; (ii) Arthritic control rats; (iii) *S. platensis* treated arthritic rats at a dose of 400 mg kg^{-1}; (iv) *S. platensis* treated arthritic rats at a dose of 800 mg kg^{-1}; and (v) Methotrexate (MTX) treated arthritic rats. All the experimental protocols were pre-approved by the institutional animal ethical committee.

Induction of collagen induced arthritis—Arthritis in the rats was induced by injecting collagen from bovine tracheal cartilage type II (Sigma) dissolved in cold 0.1*N* acetic acid (2 mg ml^{-1}) and emulsified with an equal volume of freshly opened, cold incomplete Freund's adjuvant (IFA; Sigma, USA). Rats were injected intradermally at several sites on the back (2 mg kg body weight). On the seventh day after the primary immunization, the rats were boosted with 0.1 ml (100 μg) of similarly prepared collagen/IFA emulsion injected intradermally at the base of the tail[44].

Treatment of animals with S. platensis biomass and methotrexate—The water suspension of *S. platensis* (400 and 800 mg kg^{-1}) or methotrexate (0.05 mg kg^{-1}) was administered orally to arthritic rats with the help of a syringe cannula on a daily basis from 20th to 45th day (for 25 days) after the primary immunization. Sterile water was given to the control as well as untreated arthritic control rats. The animals from various groups were first assessed both for sciatic functional index and motor coordination and subsequently immunocytochemical studies were performed to study microglial activation and neuronal degeneration.

Sciatic functional index (SFI)—For the evaluation of the SFI, first the foot prints of the animals from all the groups at various time points were collected by allowing the animals to explore a 10 cm wide and 60 cm long wooden corridor on a sheet of ink absorbing paper, with their hind paws dipped in blue ink. Every time 3-4 foot prints of each foot were marked and verniercaliper was used to take the measurements as mentioned below and the SFI was calculated and analyzed[12,45].

$$SFI = 38.3 \times \frac{EPL - NPL}{NPL} + 109.5 \times \frac{ETS - NTS}{NTS} + 13.3 \times \frac{EIT - NIT}{NIT} - 8.8$$

where NPL= Normal print length; EPL= Experimental print length; NTS= Normal toe-spread; ETS= Experimental toe-spread; NIT=Normal intermediate toe-spread; and EIT= Experimental intermediate toe-spread.

Motor coordination—Motor coordination was determined using Rotamex-5 from Columbus Instruments (USA). The instrument is completely software based with a computer interface and the data are collected in the form of time for which the animal is able to balance itself on the rotating rod. The animals were first acclimatized for 3 consecutive days with a maximum speed of 8 rpm for 100 sec. Those animals, which failed the acclimatization test were discarded and those which succeeded were allowed to run on the rotating wheel scheduled for 420 sec in a increment of 3 rpm/sec to maximum speed of 40 rpm. Three trials per animal per time point were taken at an interval of 30 min between any two trials. The data were recorded for normal controls, arthritic controls and arthritic animals treated with Spirulina or methotrexate after 5th, 10th, 15th, 20th and 25th day of treatment. The mean was calculated for further analysis and the data were analysed with one-way ANOVA using Sigma stat 3.5, Systat software.

Statistical analysis—The values are presented as mean ± SE of three rats per group. The data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey test (all pairwise multiple comparison procedure; Sigma stat 3.5, Systat software Inc. USA). A value of $P<0.05$ was considered significant to arthritic control vs. normal and treated groups.

Perfusion fixation and cryosectioning—At the end of the experiment i.e., on 45th day the animals were perfused transcardially with 0.9% buffered saline followed by cold 2% buffered paraformaldehyde. After perfusion lumbar region of the spinal cord (L_4-L_5) was carefully dissected out from the rats of various groups and post-fixed in the same fixative overnight at 4°C. Next day the tissues were washed with three changes of phosphate buffer for 30 min each and cryoprotected with sucrose gradients i.e., 10, 20 and 30% prepared in phosphate buffer at 4°C till finally settled at the bottom. Subsequently 15 µm thick sections were cut using Leica cryotome (CM1900) and collected on gelatin coated slides. The slides were stored at -20°C, till further processed for immunohistochemical study of microglial activation and neurodegeneration.

Immunocytochemistry for microglial activity—Three to four slides (each containing 5-6 sections) from each parameter were selected randomly for Iba 1 immunolabelling using streptavidin biotin HRP method. The sections were air dried at room temperature and washed in 3 changes of phosphate buffered saline (PBS). The sections were then permeabilised with 0.5%Triton X-100 in PBS for 30 min, washed in 3 changes of PBS and then treated with 1% H_2O_2 in PBS (20 min) for endogenous peroxidase blocking further followed by washing in PBS. Non-specific proteins were blocked by incubating sections in 1% normal goat serum in PBS at room temperature for 1 h and then incubated in rabbit polyclonal Iba1 antibody (Wako, Japan) at a dilution of 1:300 (diluted in 1% BSA in PBS) at 4°C overnight. Next day the sections were again washed with PBS and incubated with anti-rabbit biotin-labeled secondary antibody from Sigma (1:100; diluted with 1% BSA in PBS) at room temperature for 1 h. The sections were again washed thrice with PBS and incubated with streptavidin biotin-HRP complex (RPN 1051 from Amersham) at a dilution of 1:200 in 1% BSA in PBS for 1 h at room temperature. The sections after washing with PBS were finally visualized with 0.025% 3′3-diaminobenzidine

tetrahydrochloride and 0.03% H_2O_2 in PBS for 20 min in dark. The sections were washed with water, dehydrated with alcohol, cleared in xylene and mounted in DPX.

NF-200 immunolabelling—The cryocut sections were air dried for 1 h and washed thoroughly with PBS. Sections were permeabilized with 1% Triton X-100 in PBS for 40 min. Excess of Triton X-100 was removed by washing with PBS, three changes for 5 min each. Non-specific proteins were blocked using 3% normal goat serum in PBS for 60 min. Sections were then incubated with anti NF-200 (mouse monoclonal; Sigma) primary antibody in a moist chamber, at a dilution of 1:500 (diluted in 3% BSA in PBS) at 4°C overnight.

Next day, the sections were brought to room temperature and washed with PBS, 3 changes for 5 min each to remove any unbound primary antibody. This was followed by incubation with secondary antibody (anti-mouse TRITC labeled) at a dilution of 1:200, diluted in 3% BSA in PBS for 60 min at room temperature in dark. The sections were finally washed with 4 changes of PBS, 10 min each and mounted in aqueous non-fluorescent mounting medium from Vector (Vectashield Hardset with DAPI).The sections were visualized with Leica DM 6000 microscope fitted with a digital camera (DC 420) and filter N2.1.

Results

Development of arthritis in CIA rats—After 14-20 days of primary immunization, arthritis was developed in the rats as evident from the paw swelling, redness and ankylosis in the hind paw and ankle joints. The paw thickness (both the hind paws) was measured with the help of a vernier caliper.

Effect of S. platensis treatment on paw thickness changes in CIA rats—A gradual and significant increase in hind paw thickness due to inflammation and swelling was observed in CIA-induced arthritic control rats from 0 to 45th day with development of arthritis. This increase in paw thickness was highly significant at day 45 (P= 0.05) post arthritis induction as compared to normal control. *S. platensis* treatment given to arthritic rats both at 400 and 800 mg kg^{-1} resulted in a significant decline of paw thickness, i.e., 4.53 ± 0.15 and 4.76 ± 0.20 mm, respectively at 45th day. In contrast, the decline in paw thickness was not significant when arthritic rats were given methotrexate treatment (Table 1).

Effect of S. platensis treatment on body weight of CIA rats—There was a gradual reduction in the body weight of arthritic control rats leading to a noticeable loss in body weight on 45th day post arthritis induction as compared to the normal controls. *S. platensis* treatment to both at 400 and 800 mg kg^{-1} as well as MTX treatment to CIA rats resulted in improved body weight. However, the difference was found to be statistically non-significant (Table 1).

Effect of S. platensis treatment on sciatic functional index of CIA rats—Functional recovery assay in terms of sciatic function index (SFI) was calculated in both arthritic rats and arthritic rats treated with Spirulina or methotrexate from 5th day post treatment onwards. In arthritic animals at 45th day, the calculated SFI was -57.18, which was significantly poor as compared to that of the normal controls, i.e., -13.43. Spirulina treatment at both the doses used in the present investigation, significantly improved the SFI at all the time points. However, the best recovery was found when the arthritic animals were given Spirulina at a dose of 400 mg kg^{-1} from the 20th day of immunization to 45th day. Methotrexate treatment did not influence the SFI and as such no functional recovery was noted when arthritic animals were given MTX treatment (Table 2).

Effect of S. platensis treatment on rotarod performance of CIA rats—Motor coordination in terms of the time spent on the rotating rod was assessed in arthritic control and arthritic animals treated with Spirulina or MTX throughout the period of the study. The rotarod performance of the arthritic animals was significantly low as compared to their normal counterparts at all the time points studied. Both the *S. platensis* (400 and 800 mg kg^{-1}) and MTX treated arthritic rats showed a significant improvement in their rotarod performance in contrast

Table 1—Paw thickness and body weight in collagen induced arthritic and Spirulina and MTX-treated rats
[Values are mean ± SE]

Parameter	Normal control	Arthritic control	MTX	SP 400	SP 800
Paw thickness (mm)	4.210±0.166	5.866±0.298#	5.066±0.310	4.533±0.152*	4.766±0.202*
Body weight (g)	169.33±3.15	152±4.32	170.33±2.42	161.67±3.14	168±6.06

P= 0.05; #= significance between arthritic control vs. normal control; *= significance between arthritic control vs. Spirulina treated groups

to the untreated arthritic animals. The best performance on the rotating rod was observed in animals treated with *S. platensis* at a dose of 400 mg kg^{-1} (Table 3).

Microglial activation—Activation of microglia was distinctly apparent both in the ventral and dorsal horn of the spinal cord following induced arthritis. Microglia in the ventral horn responded more robustly than in the dorsal horn, in terms of Iba-1 expression and morphology (Fig. 1b). However, in the normal controls the microglia were in the normal ramified forms (Fig. 1a). Strongly Iba-1 expressing microglia were observed surrounding the ventral horn motor neurons. Marked downregulation in Iba-1 expression was observed after *S. platensis* treatment. *S. platensis* treated arthritic rats at both the doses, (400 and 800 mg kg^{-1}) presented clearly demarcated downregulation of Iba-1 activity. Methotrexate treatment presented no change in microglial activity in arthritic animals (Fig. 1e). *S. platensis* (400 mg kg^{-1}) treatment was found to potentially and effectively suppress the microglial activation (Fig. 1c) as compared to *S. platensis* (800 mg kg^{-1}) treatment (Fig. 1d) both in dorsal and ventral horns. Methotrexate treatment presented no influence on microglial activation in arthritis induced rat spinal cord. No visible difference was observed on glial activation between the ventral horn and dorsal horn.

NF-200 immunoreactivity—Changes in NF-200 immunoreactivity were observed in the ventral horn motor neurons of the CIA rats as well as in rats after Spirulina or methotrexate treatment as compared to the normal animals. Abnormal aggregations of neurofilaments were observed more prominently in the perikarya and axonal projections of the ventral horn neurons of CIA rats as compared to the dorsal horn of arthritic control rats. NF-200 expression was effectively downregulated after *S. platensis* treatment at a dose of 400 mg kg^{-1} in the ventral horn motor neurons. However, the methotrexate and *S. platensis* (800 mg kg^{-1}) treatments were found to be ineffective in reducing NF-200 accumulation both in the neuronal perikarya and axonal processes in the arthritic rats. However, in the entire group the result were more clearly distinguishable in large ventral horn motor neurons as compared to the small sized dorsal sensory neurons (Fig. 2a-e).

Discussion

The present study demonstrates that *S. platensis* treatment effectively counters arthritis-related pathology in experimentally-induced arthritic rats.

Table-2—Sciatic function Index (SFI) in collagen induced arthritic and Spirulina treated rats
[Values are mean ± SE]

Group	5 day	10 day	15 day	20 day	25 day
Normal Control	-11.04± 4.64	-14.82±2.72	-12.36± 4.00	+7.83± 4.07	-13.43±7.21
Arthritic control	-62.38± 1.32	-53.79±6.99	-51.10± 8.80	-42.95± 1.58	-57.18±1.92
S. platensis 400 mg/kg	+16.50± 6.01	-4.15±9.25	+ 11.99± 12.00	+14.91± 6.89	+2.73±5.74
S. platensis 800 mg/kg	-17.51±8.72	-32.25±7.10	-23.48± 16.92	-32.66± 15.64	-29.17±11.24
Methotrexate	-19.25±11.92	-48.74±5.91	-65.73± 25.54	-23.82± 24.61	-58.84±9.07

P= 0.05; *= significance between normal control vs. arthritic control;
**= significance between arthritic control vs. normal control and treated groups; # drug treated vs normal control, arthritic controls and treated groups; $ SP 800 vs SP 400 treated groups.

Table 3—Rotarod performance in collagen induced arthritic and Spirulina treated rats
[Values are mean ± SE]

Group	0 day	5 day	10 day	15 day	20 day	25 day	30 day	35 day	40 day	45 day
Normal Control	28.778 ±4.657	31.044 ±3.252	32.056 ±3.225	34.900 ±3.953	34.044 ±2.219	35.300 ±1.273	41.944 ±3.506	45.100 ±2.873	48.644 ±3.897	53.956 ±4.005
Arthritic control	26.667 ±3.424	28.189 ±3.355	28.100 ±2.620	18.100 ±2.107	6.133 ±1.388	14.100 ±1.049	12.389* ±2.267	16.211* ±2.153	16.778* ±2.207	19.078* ±2.681
S. platensis (400mg/kg)	27.389 ±3.902	28.378 ±2.575	20.967 ±2.404	11.200 ±1.947	3.822 ±1.147	33.500 ±4.451	42.978 ±3.134	42.611 ±3.247	45.422 ±2.768	44.378**@ ±2.448
S. platensis (800mg/kg)	26.689 ±3.133	29.411 ±2.963	22.033 ±1.950	12.500 ±2.578	4.822 ±1.446	36.700 ±5.306	38.811 ±3.727	39.222 ±4.799	40.478 ±1.815	40.722** ±2.373
Methotrexate	29.133 ±3.404	26.956 ±2.198	22.222 ±2.095	16.800 ±1.048	5.956 ±1.376	13.800 ±1.003	30.111 ±2.254	28.900 ±1.634	30.978 ±2.052	30.578 ±1.837

P= 0.05; *= significance between normal control vs. arthritic control; **= significance between arthritic control vs. and treated groups; @= SP 400 treated vs SP 800 or MTX treated groups

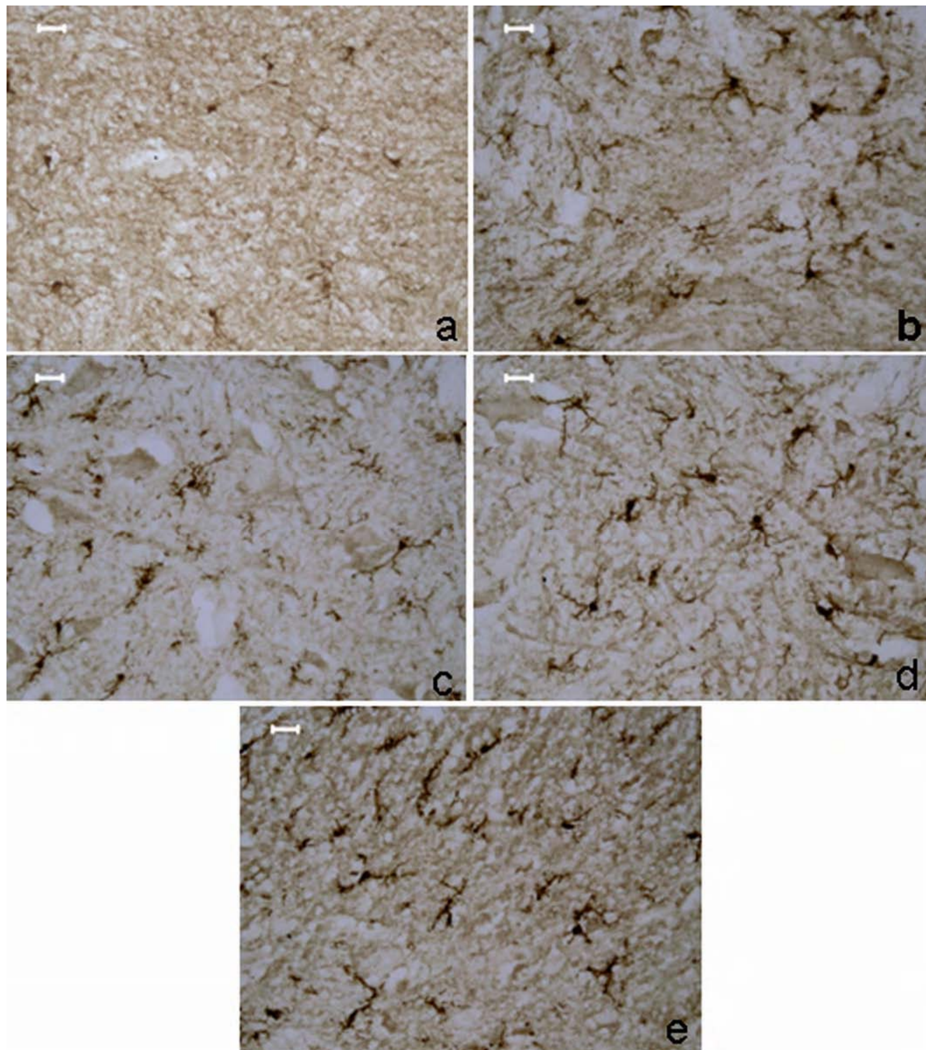

Fig. 1—Microglial activation assessed as Iba 1 expression in VH of the spinal cord. Control (a); collagen induced arthritic control (b); arthritic animals treated with *S. platensis* at 400 mg/kg body wt. dose (c); arthritic animals treated with *S. platensis* at 800 mg/kg body wt. dose (d); arthritic animals treated with methotrexate (e).

The data showed that the treatment suppresses microglial activation and prevents neurodegenerative changes in spinal motor neurons, improves motor coordination and sciatic functional index in the arthritic rats. Kumar *et al*[40]. have reported the efficacy of *S. platensis* treatment against collagen-induced arthritis on a number of athritic pathological features like: changes in paw thickness, serum albumin, cholesterol, lipid peroxidation and hydrolytic enzymes activities and the improved histology of paw joints[40]. The present study mainly focuses on changes in the neural tissue.

Inflammation of the joints is the major cause of joint pain in the arthritis that leads to the peripheral sensitization of primary sensory afferents and the development of inflammation in the dorsal horn[5]. Current therapies employed for rheumatoid arthritis have not focused on the involvement of spinal glial cells in the origin and/or maintenance of arthritis-associated pain. Thus, it is believed that any pharmacological agent that can modulate glial activation can block the exaggerated pain caused by arthritis. The support to this idea comes from the studies showing that intrathecal injection of glial metabolic inhibitor, fluorocitrate suppressed thermal hyperalgesia and metabolic allodyania evoked in arthritic rats[2,8]. Furthermore, in another study, IL-1 and TNF-α administration to healthy rodents induced hyperalgesia and allodyania, and subsequent wind up activity of the dorsal horn neurons[7]. In the present study, *S. platensis* when fed at a dose of 400 mg kg^{-1} effectively suppressed the microglial activation in

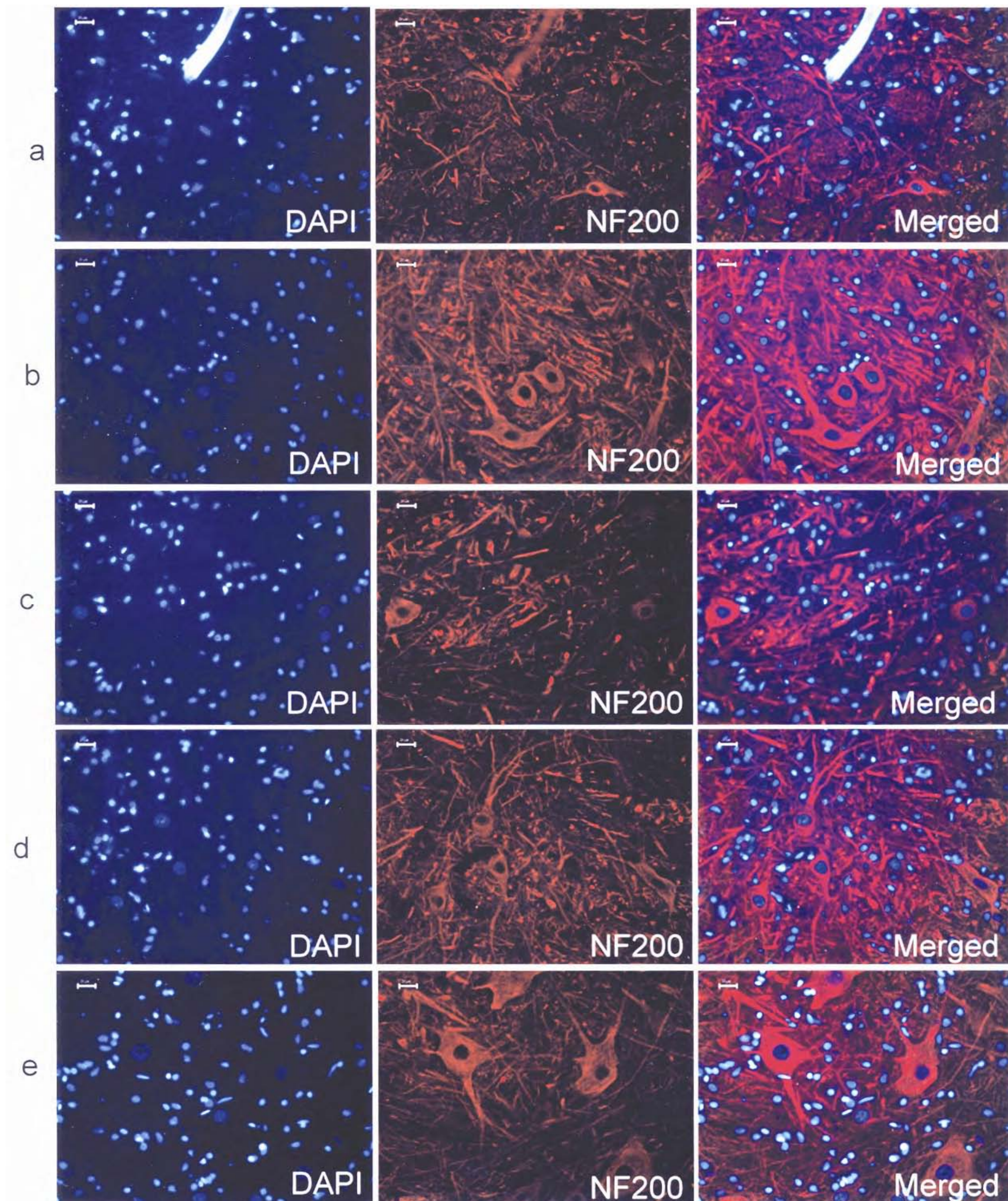

Fig. 2—NF-200 labeled neurofilament aggregation in neuronal perikarya and their processes in the motor neurons of VH of spinal cord. Control (a); collagen induced arthritic control (b); arthritic animals treated with *S. platensis* at 400 mg/kg body wt. dose (c); arthritic animals treated with *S. platensis* at 800 mg/kg body wt. dose (d); arthritic animals treated with methotrexate (e).

both the dorsal and ventral horns, and thus can be considered therapeutically effective in ameliorating central sensitization and associated pain.

Inflammatory changes in the nervous system have been variously reported to cause inflammatory pain and impaired motor function[12,46,47]. In the present study *S. platensis* has also been found to effectively suppress peripheral sensitization and improve motor coordination, and restore functional motor activity in arthritic rats. These findings

suggest that such effects may be due to *S. platensis*-induced counter regulation of spinal glial activation and thus *S. platensis* could be a potential strategy for the treatment of arthritis.

Accumulation/aggregation of aberrantly phosphorylated neurofilaments in neurons is an important feature following chemical intoxication and during many human neurodegenerative diseases[48-51]. The neurofilament heavy chain is the most exclusively phosphorylated protein in the human brain and has been variously used as a potential surrogate marker of damage to neurons and their processes[52]. NF-200 immunoreactivity was used to infer the neuronal damage following arthritis. Following collagen-induced arthritis, the abnormal accumulation of NF-200 was seen both in the neuronal perikarya and neurites of the ventral horn motor neurons as well as in the neurons of the dorsal and intermediate horns. However, the changes were more prominent in the large diameter ventral horn motor neurons. *S. platensis* treatment at both the doses used in the present study effectively lowered the NF-200 expression in the spinal cord neurons. However, comparatively the aggregation and accumulation of NF-200 was apparently much less in arthritic rats fed with the dose of *S. platensis* at 400 mg kg^{-1}. MTX treatment however, had no effect on the arthritis-induced NF-200 labeled aggregates of neurofilaments.

Aforesaid abnormal accumulation of NF-200 labeled neurofilaments may have been due to the central sensitization associated with glutamate excitotoxicity which involves calcium influx and subsequent neuronal damage in arthritic rats. Similar accumulation of phosphorylated heavy neurofilaments has also been reported in the degenerating motor neurons of the spinal cord of amyotropic lateral sclerosis (ALS) patients[53-55]. However, neurofilament dephosphorylation has also been reported to precede excitotoxicity in the spinal cord cell culture model of ALS[56,57]. Ackerley *et al.*[58] have also demonstrated in an *in vitro* study that treatment with glutamate slows axonal transport and leads to neuronal accumulation of heavy form of neurofilaments thus supporting the concept that glutamate-induced excitotoxicity may contribute to the pathogenic process in some neurodegenerative diseases[52]. The present finding that *S. platensis* treatment resulted in an appreciable reduction of the NF-200 accumulation in the spinal cord neurons of arthritic rats would suggest that *S. platensis* could be regarded as a potential neuroprotectant against the glutamate excitotoxicity induced as a result of central sensitization produced by the peripheral joint inflammation in the collagen-induced arthritis.

In conclusion, the present study clearly demonstrates that *S. platensis* effectively suppressed peripheral sensitization via modulation of glial activation, improved motor coordination, and restoration of functional motor recovery in collagen-induced arthritic rats. These findings suggest that *S. platensis* treatment could be a potential therapeutic strategy for the treatment of rheumatoid arthritis.

Acknowledgement

Thanks are due to the Department of Biotechnology, Govt. of India for financial support to Keerti Karaiya and bioinformatics infrastructural facility. *Spirulina platensis* was grown and supplied by Dr. P K Tripathi, School of Studies in Microbiology, Jiwaji University, Gwalior.

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