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Immunomodulatory effect of dietary *Spirulina platensis* in type II collagen induced arthritis in rats

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ABSTRACT

Spirulina platensis, a filamentous, multicellular microalga, is grown in certain countries as food for human and animal consumption. It is also used to derive additives in pharmaceuticals and foods. The present study was undertaken to examine the immunomodulatory effect of *S. platensis* in collagen induced arthritis (CIA) in rats. From the zero day of induction of arthritis, *S. platensis* (200 and 400 mg kg^{-1}) was administered orally to arthritic rats up to 45 days. The arthritis score, serum pro-inflammatory cytokine, anti-collagen antibody level, delayed type hypersensitivity (DTH) response to collagen and histology of paw joints were measured as marker of inflammation in arthritis. *S. platensis* significantly reduced the levels of pro-inflammatory cytokine (TNF-α, IL-1β and IL-6), arthritis score and synovial cell infiltration that had been increased in arthritic rats. Conclusion: These finding indicates that *S. platensis* had immunomodulatory effect via modulation of multiple pathways of inflammation.
Keywords: *Spirulina platensis*; Collagen induced arthritis; Rheumatoid arthritis; Inflammation; Cytokine

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INTRODUCTION

Rheumatoid arthritis (RA) is a common chronic and systemic autoimmune disorder characterized by inflammation of the synovial joints, destruction of cartilage and bone; it involves a complicated pathogenesis, with pathological changes in multiple targets [1, 2]. RA affects about 1% of the world population, in a female/male ratio of 3/1. The disease can occur at any age, its incidence increasing with age [3]. The pro-inflammatory cytokines (e.g., tumor necrosis factor alpha, interleukin-6 and interleukin-1beta) and other mediators (prostaglandins and leukotrienes) play critical roles in the development and perpetuation of tissue inflammation and damage in joint tissue such as articular cartilage and meniscus [4, 5]. The collagen induced arthritis (CIA) model in the rat is in many aspects similar to RA, which is perhaps the most commonly used model for RA today. Intradermal injection in rats with collagen emulsified in IFA leads to a severe, erosive poly-arthritis developing within 2–3 weeks after immunization followed by a subsequent chronic relapsing phase [6]. The primary drugs used in the treatment of RA are nonsteroidal anti-inflammatory (NSAIDS) and disease-modifying antirheumatic drugs (DMARDs). In most cases, these drugs have been proved to be of only limited value. They often suppress the symptoms, but accelerate factors that promote the disease. However, patients frequently become unable to continue long-term treatment with these agents due to toxicity and/or loss of benefit. Anti-TNF, anti-IL-1, and anti-IL-6 therapies have been also reported to be effective in the treatment of RA [7, 8]. Despite increased use of these combination therapies; new treatments for active RA are clearly needed.

Spirulina platensis is a blue green alga having diverse biological activity, due to high content of highly digestible proteins, vitamins, beta-carotene, phycocyanin and other pigments [9]. Early interest in *S. platensis* focused mainly on its potential as a source of protein and vitamins, but recently more attention has been made to study its therapeutic use, and a number of published reports suggest beneficial effects of this microalgae in acute allergic rhinitis [10], anti-cardiotoxic [11], anti-hepatotoxic [12] and its anti-nephrotoxic effects [13]. Keeping in view the nutritive and pharmacological properties of *S. platensis*, present investigation was undertaken to asses the immunomodulatory effect of *S. platensis* against CIA in rats.

MATERIALS AND METHODS

Experimental model

Albino female rats (Wistar strain) of 6-10 week of age were taken as the experimental animals for conducting the proposed study. CIA in rats was developed as described previously [14]. The water suspension of *S. platensis* (200 and 400 mg kg^{-1}) was administered orally on a daily basis, and the treatment was started from 0 day up to 45^{th} day. Rats were screened for the development and progression of arthritis daily from day 20^{th} to 45^{th} day. The severity of arthritis was graded as: Grade 0 = no sign of arthritis, Grade 1 = redness and swelling in paw,

Grade 2 = deformity in paw and Grade 3 = ankylosis in paw. The arthritic score of a diseased rat was sum of the maximum grades of arthritis in the involved paws. The data were expressed as mean±SE of six animals per group.

All the experimental protocols were pre-approved by the animal ethical committee, Jiwaji University, Gwalior, Madhya Pradesh, India.

Sample collection and processing

The blood samples were drawn from the retro-orbital bleeding. The blood was collected in tubes; each blood sample was centrifuged for 10 min at 5000 rpm and at 4^0C. The serum was collected and stored at – 80^0C for further investigation.

Delayed type hypersensitivity response and immunoassay of antibody to collagen

Delayed type hypersensitivity (DTH) response in rats was determined as described previously [15] at 35^{th} day. ELISA was used to detect antibodies to collagen following the method previously described [16].

Determination of TNF-α, IL- 1β and IL-6 levels in serum

Levels of pro-inflammatory cytokines TNF-α, IL- 1β and IL-6 levels in serum samples were determined by using commercially available ELISA kits specific for rat TNF-α, IL- 1β and IL-6 (Pierce Endogen, Rockford, IL, USA) according to manufacturers' recommendation.

Histopathological examination

Paw joints were collected at the end of experiment (45^{th} day) and after that tissue samples of the joints were fixed in 10% (v/v) neutral formalin and decalcified in 10% EDTA for 21 days. The decalcified tissues were dehydrated in ascending grades of alcohol and embedded in paraffin blocks, 7µ thick sections were cut with Leica RM 2135 rotary microtome and mounted on slides and stained with hematoxylin and eosin [17] to study the histopathological changes associated with CIA and *S. platensis* treatment. The stained slides were visualized using a Leica DM 6000 (Germany) equipped with digital camera and the images were captured using a Leica Application Suite software.

Statistical analysis

The values were presented as mean±SE of six rats per group. Results were analyzed by one-way analysis of variance (ANOVA) followed by Tukey test (all pair wise 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.

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RESULTS

Effect of dietary *S. platensis* on arthritic score, delayed type hypersensitivity response and IgG antibody titer

A significant increment in arthritic score, serum anti-collagen IgG antibody and DTH response was observed in arthritic control rats. However, *S. platensis* (400 mg kg^{-1}) treatment to the arthritic rats resulted in a significant decline in their arthritic score as compared to their arthritic control counterparts. *S. platensis* treatment to the arthritic rats also showed decline in their serum anti-collagen IgG antibody level and DTH response, however, the decrease in serum anti-collagen IgG antibody level and DTH response was found to be statistically non significant (Fig. 1).

Effect of dietary *S. platensis* on serum TNF-α, IL-1β and IL-6 levels

A significant elevation in serum TNF-α, IL-1β and IL-6 concentration from 0 day to 45^{th} day was recorded in arthritic control rats. *S. platensis* (400 mg kg^{-1}) treatment to the arthritic rats resulted in a significant decline in their serum TNF-α, IL-1β and IL-6 concentration at 30^{th} and 45^{th} day as compared to their arthritic control counterparts (Fig. 2).

Effect of dietary *S. platensis* on histopathology of paw joints

Microscopic analysis of phalangial joint articulations showed that the joint of arthritic control rats had moderate to severe synovitis, considerable inflammatory cell infiltration into mineralized and non-mineralized tissues. *S. platensis* (200 mg kg^{-1}) treated arthritic rats had moderate synovitis, minimal to moderate inflammatory cell infiltration into tissues, and no signs of cartilage ulceration. In arthritic rats treated with *S. platensis* (400 mg kg^{-1}), synovitis and inflammatory cell influx was minimal, with no sign of cartilage ulceration (Fig. 3).

DISCUSSION

RA is a systemic inflammatory disorder that mainly affects the diarthrodial joint. It is the most common form of inflammatory arthropathy worldwide and affects up to 0.75% of the Indian population [18]. The spectrum and disease progression of RA is governed by multiple factors including immune, genetic and environmental factors [19, 20]. Rodent models of RA serve as valuable tools to investigate the underlying mechanisms at early, intermediate and late stages of RA [21, 22]. The goal of generating new and improved treatments for arthritic diseases of the joints has not yet been achieved, although progress is being made. Destruction of articular cartilage is the hallmark of many of these disabling conditions, and an imbalance of pro-inflammatory cytokines over their anti-inflammatory counterparts promotes the disease process. CIA in rodents, an experimental disease model with a number of pathological,

histological, immunological and genetic parameters common with RA was used in the present study [23].

Many investigators have shown that cytokines play essential roles in the pathogenesis of CIA. In particular, inflammatory cytokines, such as IL-1β and TNF-α, are considered to be key cytokines, on the basis of the finding that anti- IL-1β and anti-TNF-α therapies suppress the development of CIA [24, 25]. In animal experiments, serum TNF-α, IL-1β and IL-6 are recorded significantly higher in CIA in rats [26, 27, 28]. In the present study, a significant elevation in serum TNF-α, IL-1β and IL-6 concentration was observed in arthritic control rats during the development of arthritis. It also showed statistically significant difference as compared to their normal rats. However, *S. platensis* (400 mg kg^{-1}) treatment to the arthritic rats resulted in a significant decline in their serum TNF-α, IL-1β and IL-6 concentration as compared to the TNF-α, IL-1β and IL-6 concentrations of their arthritic control counterparts.

S. platensis is considered a valuable additional food source with some macro and micronutrients including high quality protein, phycocyanin, iron, gamma-linolenic fatty acids, cartenoids and vitamins [29]. Dietary *Spirulina* also showed modulation of immunological functions in animal experiments [30]. In previous studies it is found that phycocyanin from *Spirulina* reduced the TNF -α and nitrite levels in serum and liver inductible nitric oxide synthase (iNOS) activity in rats subjected to thyroid hormone (T_3) administration [31]. In an extensive study it is found that *Spirulina* inhibited anaphylactic shock 100% with doses of 0.5 and 1.0 mg g^{-1} body weight and had a significant effect on the anti-dinitrophenyl IgE-induced histamine release or TNF-α production from rat peritoneal mast cells [32]. In another study, rats treated with intra-peritoneal dosages of *Spirulina* at concentrations ranging from 0.01 to 1,000 µg g^{-1} body weight, serum histamine levels were reduced in a dose dependent manner. *Spirulina* also had a significant inhibitory effect on anti-dinitrophenyl IgE -induced TNF-α production [33]. Our findings are supported by these above studies.

In our opinion, the mechanism by which *S. platensis* exerts its anti-chemoattractant action on synovial cell infiltration or pannus formation in joints may be due to its inhibitory effects on the biosynthesis of the neutrophil chemotactic mediator leukotriene B4 (LTB4). This view is also supported by previous findings that phycocyanin from *Spirulina* reduced LTB4 levels in the arachidonic acid induced mouse ear inflammation test [34]. Our finding are also supported by Remirez *et al.* [35] where *Spirulina* treatment revealed a marked decrease of histology score, the inflammatory reaction was substantially reduced and there was no destruction of joint architecture or pannus formation and a reduction in bone erosion was observed in zymosan induced arthritis in mice [35].

CONCLUSION

The present results indicate that oral administration of *S. platensis* to CIA rats significantly reduced the levels of pro-inflammatory cytokine, arthritis score and synovial cell

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infiltration that had been increased in arthritic rats. Taking into account of the above findings, it can be concluded that the potential immunomodulatory effects of *S. platensis* in type II collagen induced arthritis in rats may be due to antioxidant and anti-inflammatory activities of its constituent phycocyanin. The results of the present study also indicate that *S. platensis* administration in the preventative protocol can significantly alter the progression of collagen-induced arthritis in rats. But precaution should be taken when translating the findings from the current study to clinical situation; we believe that *S. platensis* require further pre-clinical and clinical studies to determine its place as potential immunomodulatory drug for the treatment of rheumatoid arthritis and other inflammatory diseases.

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Fig. 1 Effect of *Spirulina platensis* treatment on arthritic score, delayed type hypersensitivity (DTH) response and IgG antibody titre in CIA rats. (A) Arthritic score, (B) DTH response and (C) IgG antibody titer. Data are expressed as mean ± standard error of six rats per group.
*** P<0.05 & # P<0.001 arthritic control vs. normal and treated groups.**

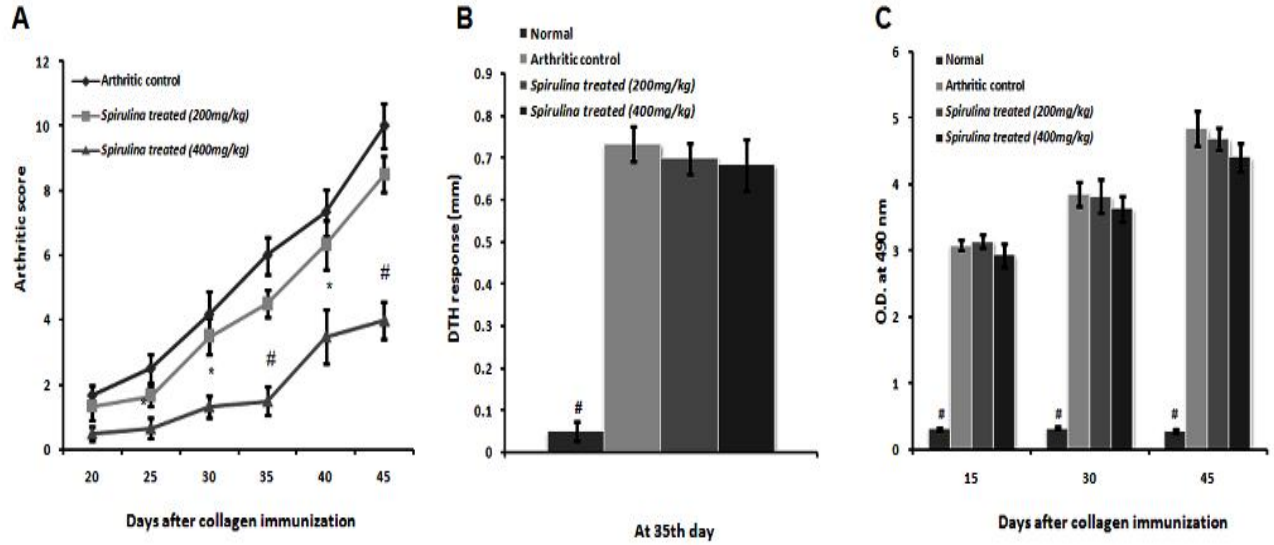

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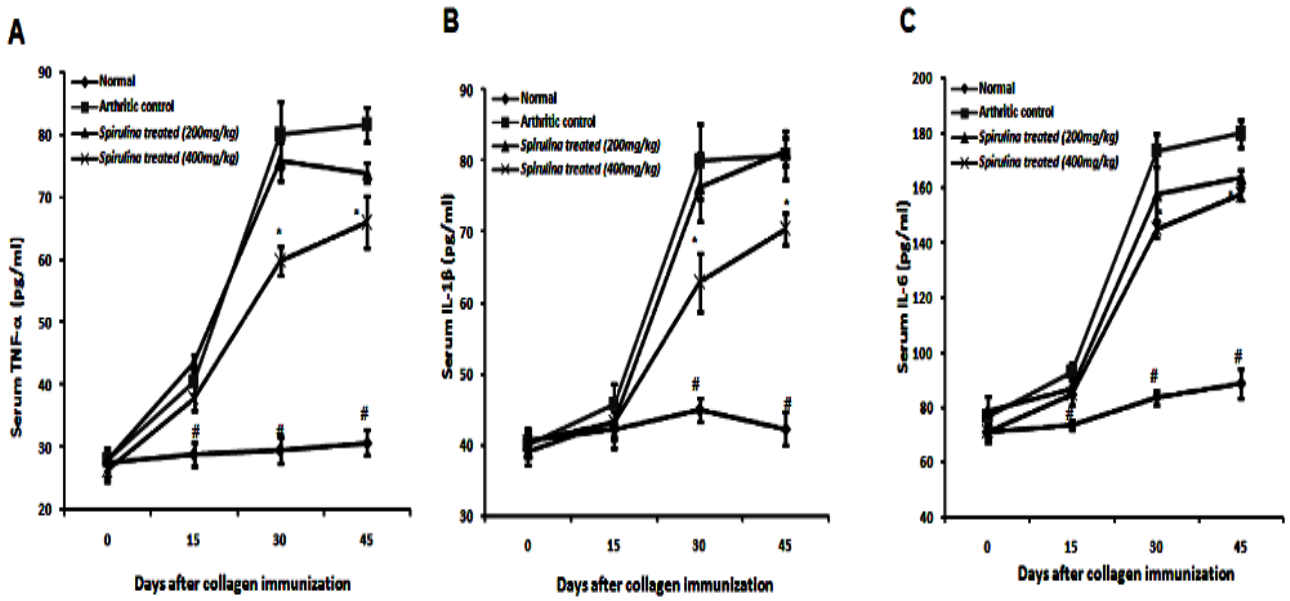
Fig. 2 Effect of *Spirulina platensis* treatment on serum cytokines in CIA rats (A) TNF-α (B) IL-1β and (C) IL-6. Data are expressed as mean ± standard error of six rats per group. * P<0.05 & # P< 0.001 arthritic control vs. normal and treated groups.



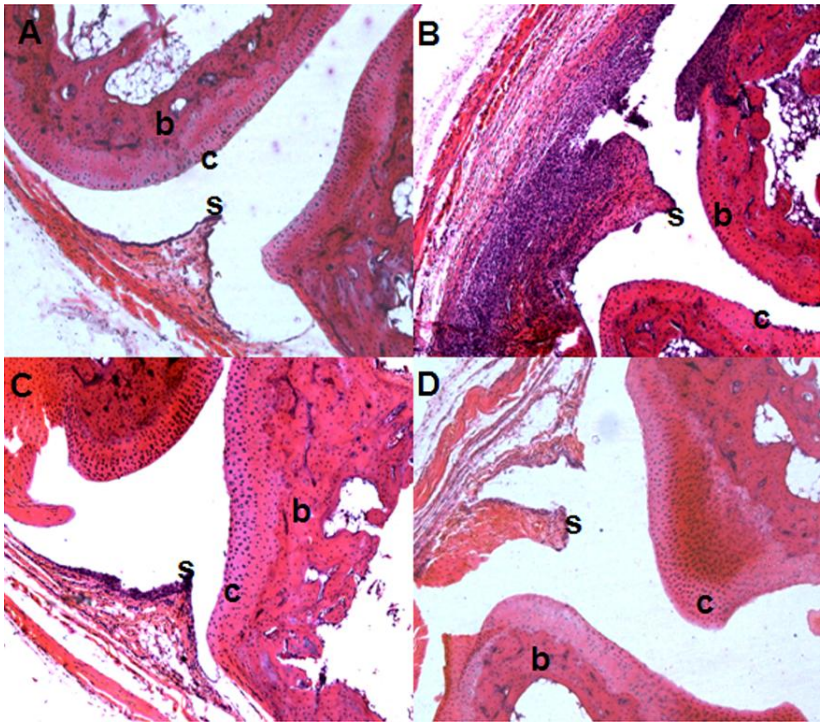


Fig. 3 Light micrographs (500X) of rat phalangial articulations after hematoxylin and eosin staining, (A) Normal rat (B) Arthritic control rat (C) *Spirulina platensis* (200 mg kg^{-1}) treated rats (D) *S. platensis* (400 mg kg^{-1}) treated rats. b, bone; c, cartilage; s, synovium. Pictures are representative of six distinct rats per group (colour figure online).

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