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Suppressive effect of *Spirulina fusiformis* in relation to lysosomal acid hydrolases, lipid peroxidation, antioxidant status, and inflammatory mediator TNF-alpha on experimental gouty arthritis in mice

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SUMMARY

The anti-inflammatory effect of *Spirulina fusiformis* on monosodium urate crystal-induced inflammation in mice has been investigated and compared with the non-steroidal anti-inflammatory drug Indomethacin. The paw volume, lysosomal enzyme activities, lipid peroxidation, anti-oxidant status and inflammatory mediator tumour necrosis factor-α were studied in control and monosodium urate crystal-induced mice after oral administration of Spirulina platensis in an experimental model for gouty arthritis. In the induced mice, the levels of lysosomal enzymes, inflammatory mediator tumour necrosis factor-α, lipid peroxidation and the paw volume increased significantly, whereas the antioxidant status decreased when compared to control mice. β-glucuronidase and lactate dehydrogenase level were also found to be increased in untreated monosodium urate crystal-incubated polymorphonuclear leucocytes. After the oral administration of *Spirulina fusiformis*, the physical and biochemical changes observed in monosodium urate crystal-induced animals were significantly restored to near normal levels. The results clearly indicated the anti-inflammatory role of *Spirulina fusiformis,* a promising drug for gouty arthritis.

Key words: *Spirulina fusiformis;* Inflammation; Tumour necrosis factor-α; Gouty arthritis

INTRODUCTION

Gouty arthritis is characteristically an intense acute inflammatory reaction that erupts in response to articular deposits of monosodium urate crystals in leucocytes. Clinically, gouty arthritis is associated with aedema and erythema of joints, together with severe pain. During such attacks, monosodium urate crystals are found in leukocytes in synovial fluids, indicating their involvement in acute arthritis (Martinon *et al*., 2006). In a previous study (Inokuchi *et al*., 2006), it has been reported that monosodium urate crystals stimulate synovial cells, monocytes-macrophages, and neutrophils to produce different types of cytokines like tumor necrosis factor (TNF)-α, interleukin (IL)-8, IL-1β, IL-6, and monocyte chemotactic factor, which induce acute inflammation. For controlling such acute gout condition non-steroidal anti-inflammatory drugs such as Indomethacin, naproxen and oral colchicine are frequently used as

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first-line therapies. However, they induce predictable gastrointestinal side effects, as they are poorly tolerated by humans. Therefore, the search for alternate herbal drugs.

Spirulina, a blue green algae, has been used since ancient times as a source of food because of its high protein and nutritional value. The chemical composition of Spirulina indicates that it has phenolic acids, tocopherols and β-carotene, which are known to exhibit antioxidant properties (Seshadri *et al*., 1991). *Spirulina fusiformis* possess potent antiviral activity, anticancer effects, strengthens immune system, and metalloprotective effects (Premkumar *et al*., 2004). Its safety for human consumption has also been established through numerous toxicological studies (Tomohiro Hirahashi *et al*., 2002). Moreover in our laboratory, we have already reported that the *Spirulina fusiformis* possess anti-inflammatory effect against adjuvant-induced arthritis in mice (Mahaboobkhan Rasool *et al*., 2006). By considering its nutritional value and our earlier reports, this study was undertaken to examine the anti-inflammatory effect of *Spirulina fusiformis* against monosodium urate crystal-induced inflammation in mice; an experimental model for gouty arthritis. The standard non-steroidal anti-inflammatory drug, Indomethacin, was used as a reference drug for purposes of comparison.

MATERIALS AND METHODS

Animals

Swiss albino mice, 25 - 30 g, of either sex were obtained from the Tamil Nadu Veterinary College, Chennai, India. They were acclimatized for a week in a light and temperature –controlled room with a 12 h dark-light cycle and fed commercial pelleted feed (Water 8.9%, Protein 25.4%, Lipid 4.4%, Carbohydrate 50.3%, Ash 6.9%, and Crude fiber 4.1%) from Hindustan Lever Ltd. (Mumbai, India) and water was freely available. Animals used in this study were treated and cared for in accordance with the guidelines recommended by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India, Ministry of Culture, Chennai. Experimental protocol was approved by School of Biotechnology, Chemical and Biomedical engineering; VIT University; Animal house ethical committee.

Drugs

The commercially available *Spirulina fusiformis* (a fine dark blue-green spray-dried powder) was obtained from RECON, Ltd, Bangalore, India and was dissolved in 2% gum acacia solution to give an aqueous suspension. The phytochemical analysis of *Spirulina fusiformis* showed the presence of total carotenes 4 mg g^{-1}, β-carotene 2 mg, vitamin B complex 0.06 mg, cyanocobalamin 1 µg, iron 1 mg, phosphorus 8.5 mg, zinc 0.35 mg, potassium 13.5 mg , water 9%, protein 55%, ash 9%, and fiber 0.9%. This aqueous suspension of *Spirulina fusiformis* was used at a dose of 800 mg/kg/b.wt orally. Indomethacin (Tamilnadu Dadha Pharmaceuticals, Chennai, India) was dissolved in 2% gum acacia solution and 3 mg/kg/b.wt was administered orally (MahaboobKhan Rasool and Palaninathan Varalakshmi, 2006). All other reagents used were standard laboratory reagents of analytical grade and were purchased locally.

Dosage

Based on our preliminary studies with different dosages (250 mg, 400 mg, 600 mg, 800 mg) of *Spirulina fusiformis*, it was found that the 800 mg/kg b.w dosage produced a significant anti-inflammatory effect by reducing paw swelling in monosodium urate crystal-induced animals. Hence the 800 mg/ kg b.w dosage was used for this study.

Synthesis of monosodium urate crystals

About 4 g of uric acid was dissolved and heated in 800 ml H_2O with NaOH (9 ml/0.5 N), adjusted to pH 8.9 at 60 °C; cooled over night in a cold room; washed and dried. Needle-like crystals were recovered which were suspended in sterile saline (MahaboobKhan

Rasool and Palaninathan Varalakshmi, 2006).

Experimental design

The mice were divided into four groups-each comprised of six animals. Group I served as controls. In Group II, inflammation was induced by intradermal injection of 0.2 ml (4 mg) of monosodium urate crystal suspension into the right foot pad. Group III and Group IV comprised of monosodium crystal-induced mice were orally administered with Spirulina fusiformis (800 mg/kg body weight) and Indomethacin (3 mg/kg body weight) respectively, 1 h before the monosodium urate crystal injection and which was repeated for 3 more days on a daily basis.

The inflammation was quantified by measuring the thickness of the paw with vernier scale at different intervals for 3 days. At the end of the experimental period (72 h), the mice were killed by cervical decapitation. Blood from each animal was collected for serum separation. The liver and spleen were immediately dissected out and homogenized in ice-cold 0.01 M, Tris HCL buffer, pH 7.4 to give a 10% homogenate. The tissue homogenate of spleen, liver and serum were used for assaying the lysosomal enzymes, lipid peroxidation, antioxidant status and inflammatory mediator tumour necrosis factor-α respectively.

Effect of *Spirulina fusiformis* on lysosomal enzymes

The activity of acid phosphatase was assayed by the method of King (1965a). β-glucuronidase was assayed by the method of Kawai and Anno (1971) and the activity of β-galactosidase was assessed by the method of Rosenblit (1974). The method of Marhun (1976) was followed for the determination of *N*-acetyl glucosaminidase and the protein content was measured by the technique of Lowry *et al*. (1951).

Effect of *Spirulina fusiformis* on lipid peroxidation and antioxidant status

Lipid peroxidation in plasma was estimated by the method of Ledwozy *et al.* (1986). Spleen and liver lipid peroxidation was carried out by the procedure of Hogberg *et al.* (1974) using thiobarbutric acid (TBA) as the colouring agent. Malonaldehyde (MDA) produced during peroxidation of lipids served as an index of lipid peroxidation. MDA reacts with TBA to generate a colour product, which absorbs at 532 nm.

Superoxide dismutase (SOD) activity in spleen and liver was determined by the method of Marklund and Marklund (1974). The degree of inhibition of the auto-oxidation of pyrogallol at an alkaline pH by Superoxide dismutase was used as a measure of the enzyme activity. Catalase and glutathione peroxidase activities in the spleen were estimated by the method of Sinha (1972) and Rotruck *et al.* (1973). The activity of catalase was expressed as μg of H_20_2 consumed/mn/mg protein. Glutathione peroxidase was expressed as μg of glutathione utilized/minute/mg/protein.

Effect of *Spirulina fusiformis* on TNF-α production

TNF-α levels in plasma, liver and spleen of the control and monosodium urate crystal induced mice were determined by enzyme-linked immunosorbent assay (ELISA, Cayman Chemicals Company, USA), according to the manufacturer's instruction.

***In vitro* studies (monosodium urate crystal-polymorphonuclear leucocyte cell interaction)**

Human polymorphonuclear leucocyte cell suspension (3×10^6 ml^1) was pre-incubated at 37 °C for 20 min with Spirulina fusiformis (100 μg/ml) or Indomethacin (10 μg/ml) before addition of monosodium urate crystals (1 mg/ml). After incubation for a further 30 min at 37 °C, the cell suspension was removed and centrifuged at 1500 × g at 40 °C for 20 min. The resultant cell free supernatant was assayed for the released activities of β-glucuronidase and lactate dehydrogenase. Appropriate control experiments were performed by measuring the release of enzymes tested in the untreated specimens and those incubated for 30 min at 37 °C without the

drug. In all instances, the experiments were carried out in triplicate.

Lactate dehydrogenase (LDH), a cytoplasmic enzyme was assayed by the method of King (1965b). β-glucuronidase, an enzyme present in azurophilic granules was measured by the method of Kawai and Anno (1971). Enzyme released was expressed as a percentage of maximal enzyme release after disruption of the cells with 0.2 Triton X-100. Specific enzyme activity was expressed as units/mg of protein.

Statistical analysis

The results were expressed as mean ± S.D. Both *in vivo* and *in vitro* studies were analysed statistically using one way ANOVA to determine the significant differences between the control and experimental groups, followed by Student's Newman-Keul's test by using SPSS Software (Student's version). The minimum level of significance was set at $P < 0.05$.

RESULTS

Effect of *Spirulina fusiformis* on lysosomal enzymes

Table 1 depicts the activity of *Spirulina fusiformis* on lysosomal enzymes in the plasma, liver and spleen of control and experimental animals. Levels of acid phosphatase, β-glucuronidase, N-acetyl glucosaminidase and β-galactosidase were increased significantly in plasma, liver and spleen of monosodium urate crystal-induced mice compared to control mice. The administration of *Spirulina fusiformis* to

Table 1. Effect of *Spirulina fusiformis* and Indomethacin on the activities of lysosomal enzymes in monosodium crystal-induced mice

Parameter	Group–I (Control)	Group–II (Monosodium urate crystal-induced mice)	Group–III (Monosodium urate crys-tal-induced mice + *Spirulina fusiformis* 800mg/kg/b.wt)	Group–IV (Monosodium urate crystal-induced mice + Indomethcin 3 mg/kg/b.wt)
Serum				
Acid phosphatase	0.15 ± 0.01	0.32 ± 0.02a*	0.19 ± 0.01a*b*	0.21 ± 0.02 a*b*
β-glucuronidase	2.10± 0.13	5.10 ± 0.39a*	2. 70 ± 0.15a*b*	2.65 ± 0.16a*b*
N-acetyl glucosaminidase	1.10 ± 0.09	2.55 ± 0.19a*	1.25 ± 0.1a*b*	1.29 ± 0.09a*b*
β-galactosidase	2.05 ± 0.07	3.15 ± 0.28a*	2.28 ± 0.12a*b*	2.40 ± 0.17a*b*
Liver				
Acid phosphatase	2.12 ± 0.16	3.61 ± 0.22a*	2. 6 ± 0.15a*b*	2.65 ± 0.23a*b*
β-glucuronidase	25.5 ± 1.82	35.7 ± 2.97a*	27.5 ± 2.25a*b*	29.5 ± 2.55a*b*
N-acetyl glucosaminidase	28.4 ± 2.36	40.5 ± 3.11a*	31.7 ± 2.75a*b*	32.0 ± 2.60a*b*
β-galactosidase	10.9 ± 0.68	18.8 ± 1.10a*	13.5 ± 0.75a*b*	13.2 ± 0.85a*b*
Spleen				
Acid phosphatase	2.9 ± 0.22	3.8 ± 0.24a*	3.35 ± 0.27a*b*	3.1 ± 0.28a*b*
β-glucuronidase	26.7 ± 1.92	37.5 ± 2.08a*	28.5 ± 1.67a*b*	30.5 ± 2.60a*b*
N-acetyl glucosaminidase	20.5 ± 1.57	35.4 ± 1.96a*	24.7 ± 1.37a*b*	25.7 ± 1.97a*b*
β-galactosidase	4.6 ± 0.35	10.5 ± 0.62a*	5.5 ± 0.32a*b*	5.3 ± 0.37a*b*

Treatment of groups are as follows: Group I- Control mice; Group II –Monosodium urate crystal-induced mice; Group III- Monosodium urate crystal-induced mice treated with *Spirulina fusiformis* (800 mg/kg/b.wt); Group IV- Monosodium urate crystal-induced mice treated with Indomethacin (3 mg/kg/b.wt).Values are expressed as mean±S.D. of six animals. Comparisons were made as follows: a-Group-1 vs. Groups-II, III, and IV b-Group-II vs. Group-III, and IV Enzyme activities are expressed as: Acid phosphatase–μ moles × 10^{-2} of phenol; β-glucuronidase, N-acetyl glucosaminidase and β-Galactosidase-μ moles × 10^{-2} of p-nitro phenol liberated/h/mg protein. *Indicate statistically significance at: $P < 0.05$. (.Statistical analysis was calculated by one way ANOVA followed by Student's Newman-Keul's test)

Table 2. Effect of *Spirulina fusiformis* and Indomethacin on lipid peroxidation in monosodium urate crystal-induced mice

Parameter	Group-I (Control)	Group II (Monosodium crystal-induced mice)	Group III (Monosodium urate crystal-induced mice + *Spirulina fusiformis* 800mg/kg/b.wt)	Group IV (Monosodium urate crystal-induced mice+Indomethacin 3mg/kg/b.wt)
Plasma	3.15 ± 0.19	5.01 ± 0.24a*	4.12 ± 0.19a*b*	4.2 ± 0.26a*b*
Liver	1.53 ± 0.10	2.12 ± 0.15a*	1.58 ± 0.10b*	1.55 ± 0.17b*
Spleen	2.15 ± 0.17	3.55 ± 0.27a*	2.45 ± 0.14a*b*	2.65 ± 0.20a*b*

Group I-Control mice; Group II–Monosodium urate crystal-induced mice; Group III-Monosodium urate crystal-induced mice treated with *Spirulina fusiformis* (800mg/kg/b.wt); Group IV- Monosodium urate crystal-induced mice treated with Indomethacin (3 mg/kg/b.wt). Values are expressed as mean±S.D. of six animals. Comparisons were made as follows: a-Group-1 vs. Groups-II, III, and IV b-Group-II vs. Group-III, and IV. Values are expressed as: nanomoles of malonaldehyde formed/mg protein; plasma-mg/dl. *Indicate statistically significance at: $P < 0.05$. (Statistical analysis was calculated by one way ANOVA followed by Student's Newman-Keul's test)

monosodium urate crystal-induced mice significantly reversed the above changes to a normal level.

Effect of *Spirulina fusiformis* on lipid peroxidation

Table 2 shows the effect of *Spirulina fusiformis* on lipid peroxidation in plasma, liver and spleen of control and experimental mice. In Group II monosodium urate crystal-induced mice, lipid peroxide level in plasma, liver and spleen was increased significantly compared to control group, whereas administration of *Spirulina fusiformis* to monosodium urate crystal-induced mice altered the above changes by regulating the lipid peroxide level to nearly that of normal levels.

Effect of *Spirulina fusiformis* on antioxidant status

Table 3 shows the effect of *Spirulina fusiformis* on the enzymic antioxidant levels in liver, and spleen

Table 3. Effect of *Spirulina fusiformis* and Indomethacin on enzymic antioxidant status in monosodiumurate crystal-induced mice

Parameter	Group-I (Control)	Group II (Monosodium crystal-induced mice)	Group III (Monosodium urate crystal-induced mice + *Spirulina fusiformis* 800mg/kg/b.wt)	Group IV (Monosodium urate crystal-induced mice + Indomethacin 3 mg/kg/b.wt)
Liver				
Superoxide dismutase	3.4 ± 0.2	1.9 ± 0.11a*	2.90 ± 0.23a*b*	2.8 ± 0.25a*b*
Glutathione peroxidase	7.78 ± 0.64	4.35 ± 0.27a*	6.65 ± 0.61a*b*	6.75 ± 0.45a*b*
Catalase	12.1 ± 0.75	6.75 ± 0.48a*	10.8 ± 0.70a*b*	10.5 ± 0.7a*b*
Spleen				
Superoxide dismutase	2.25 ± 0.16	1.5 ± 0.12a*	2.04 ± 0.16b*	1.95 ± 0.14a*b*
Glutathione peroxidase	4.5 ± 0.28	3. 2 ± 0.18a*	4.35 ± 0.35b*	4.25 ± 0.26b*
Catalase	9.5 ± 0.73	5.4 ± 0.38a*	9.05 ± 0.72a*b*	8.3 ± 0.55a*b*

Group I-Control mice; Group II–Monosodium urate crystal-induced mice; Group III-Monosodium urate crystal-induced mice treated with *Spirulina fusiformis* (800 mg/kg/b.wt); Group IV- Monosodium urate crystal-induced mice treated with Indomethacin (3 mg/kg/b.wt). Values are expressed as mean±S.D. of six animals. Comparisons were made as follows: a-Group-1 vs. Groups-II, III, and IV b-Group-II vs. Group-III, and IV. Enzyme units are expressed as; SOD-units/mg protein (unit-amount of enzyme required to inhibit the auto-oxidation reaction by 50%); Glutathione peroxidase-μg of GSH utilised/min/mg protein; Catalase-μmol of H_20_2 consumed/min/mg protein. *Indicate statistically significance at: $P < 0.05$. (Statistical analysis was calculated by one way ANOVA followed by Student's Newman-Keul's test).

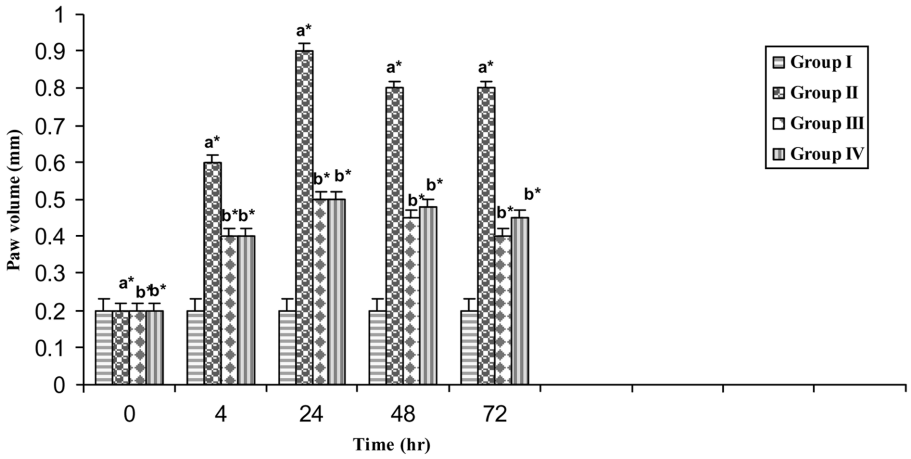

Fig. 1. Effect of *Spirulina fusiformis* and Indomethacin on paw oedema in monosodium urate crystal-induced mice. Group I: control mice; Group II: monosodium urate crystal-induced mice; Group III: *Spirulina fusiformis* (800 mg/kg body weight) treated monosodium urate crystal-induced mice; Group IV: Indomethacin (3 mg/kg body weight) treated monosodium crystal-induced mice. Values are expressed as mean ± S.D. (n = 6). Comparisons are made with a: Group I vs. Group II, III, and IV, b: Group II vs. Groups III and IV. *Indicate statistically significance at: P<0.05. (Statistical analysis was calculated by one way ANOVA followed by Student's Newman-Keul's test).

of control and experimental mice. The enzymic antioxidants were significantly decreased in monosodium urate crystal-induced mice compared to control mice. The administration of *Spirulina fusiformis* increased the enzymic antioxidant levels in monosodium urate crystal-induced mice considerably, which indicates its antiperoxidative action.

Anti-inflammatory activity of *Spirulina fusiformis*

Figure 1 shows the effect of *Spirulina fusiformis* on paw oedema in control and experimental animals. The measurement of the paw volume of monosodium urate crystal induced mice revealed an increase in ankle diameter. *Spirulina fusiformis* treatment reduces the paw diameter significantly in monosodium urate crystal-induced mice.

***In vitro* studies (monosodium urate crystal-polymorphonuclear leucocyte cell interaction)**

Figure 2 shows the effect of Spirulina fusiformis and Indomethacin on the β-glucuronidase (lysosomal) and lactate dehydrogenase (cytoplasmic) enzyme

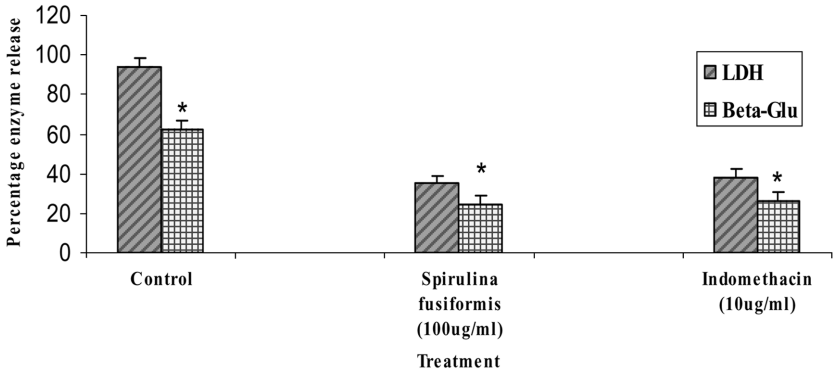

Fig. 2. Effect of *Spirulina fusiformis* and Indomethacin on enzyme leakage from the polymorphonuclear lecucocyte cells (PMNL) upon incubation with monosodium urate crystals. Values are expressed as mean ± S.D. (n = 6). Comparisons are made with control groups. *Indicate statistically significance at: P < 0.05. (Statistical analysis was calculated by one way ANOVA followed by Student's Newman-Keul's test).

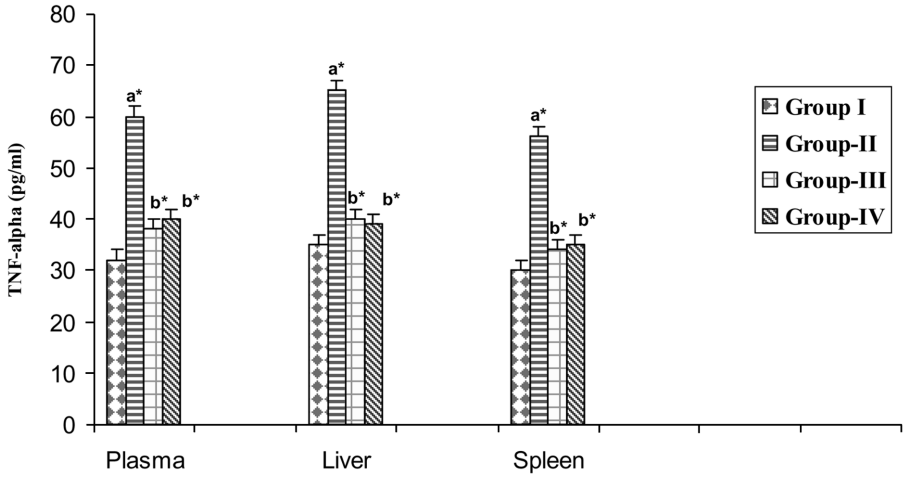

Fig. 3. Effect of *Spirulina fusiformis* and Indomethacin on TNF-α production in monosodium urate crystal-induced mice. Group I: control mice; Group II: monosodium urate crystal-induced mice; Group III: *Spirulina fusiformis* (800 mg/kg body weight) treated monosodium urate crystal-induced mice; Group IV: Indomethacin (3 mg/kg body weight) treated monosodium crystal-induced mice. Values are expressed as mean ± S.D. (n = 6). Comparisons are made with a: Group I vs. Group II, III, and IV, b: Group II vs. Groups III and IV. *Indicate statistically significance at: $P < 0.05$. (Statistical analysis was calculated by one way ANOVA followed by Student's Newman-Keul's test).

release from the polymorphonuclear leucocytes cells incubated with monosodium urate crystals. The pretreatment of *Spirulina fusiformis* (100 μg/ml) significantly suppresses the lysosomal and cytoplasmic enzyme release from the polymorphonuclear leucocytes cells incubated with monosodium urate crystals compared to controls, i.e. untreated polymorphonuclear leucocytes cells incubated with monosodium urate crystals.

Effect of *Spirulina fusiformis* on TNF-α production

Figure 3 shows the levels of pro-inflammatory cytokine tumour necrosis factor-α in the serum, liver and spleen of control and experimental animals. Levels of tumour necrosis factor-α in the monosodium urate crystal mice were systemically overproduced in the serum, liver and spleen; while the elevated levels of tumour necrosis factor-α were found to be decreased in Spirulina fusiformis treated monosodium urate crystal-induced mice.

DISCUSSION

In the present study, we have investigated the anti-inflammatory effect of *Spirulina fusiformis,* in monosodium urate crystal-induced inflammation in mice; an experimental model for gouty arthritis. *Spirulina fusiformis* significantly inhibited the paw volume and the levels of lysosomal enzymes, lipid peroxidation, and inflammatory mediator tumour necrosis factor-α; whereas it restored the antioxidant status to a near normal level in monosodium urate crystal-induced mice compared to control mice. In addition, *Spirulina fusiformis* treatment decreased the lysosomal and cytoplasmic enzyme release from the monosodium urate crystal-incubated polymorphonuclear leucocyte cells.

In our study, the activities of lysosomal enzymes in plasma, liver, and spleen of arthritic mice were elevated as compared to control group. Since, extracellular release of lysosomal enzymes may be crucial to the pathogenesis of tissue injury and inflammation, it is likely that a reduction in the release of such enzymes would prove beneficial. A decrease in lysosomal stability is generally paralleled by increased lysosomal enzyme activity in extra cellular fluid (Gebbia *et al.*,1985). The reduction in the paw edema and lysosomal enzyme activities

after oral administration of *Spirulina fusiformis* suggests that this drug can inhibit the release of lysosomal enzymes by its stabilizing action.

In a number of pathophysiological conditions associated with inflammation or oxidant stress, reactive oxygen species (ROS) have been proposed to mediate cell damage via a number of independent mechanisms including (i) the initiation of lipid peroxidation, and (ii) the inactivation of a variety of enzymes (Cuzzocrea *et al*., 1998). In order to better understand the mechanisms of the observed anti-inflammatory effects of *Spirulina fusiformis*, we have also investigated whether *Spirulina fusiformis* attenuates the levels of lipid peroxide, hydroxy radical and superoxide dismutase activity observed in monosodium urate crystal-induced mice.

The increased lipid peroxide level noticed in monosodium urate crystal-induced mice in our study may be due to its release from neutrophils and monocytes during inflammation (MahaboobKhan Rasool and Palaninathan Varalakshmi, 2006). Our results show that the activities of superoxide dismutase, glutathione peroxidase and catalase are found to be decreased in monosodium urate crystal-induced animals which may be due to enormous production of free radicals. After the *Spirulina fusiformis* treatment, the alterations produced in monosodium crystal-induced mice with respect to lipid peroxidation and antioxidants concentrations were modulated to near normal levels. This modulating role of *Spirulina fusiformis* may be due to antiperoxidative action of its components that were observed earlier in other studies (Premkumar *et al*., 2001). The active components found in *Spirulina* may provoke the activity of free radical scavenging enzyme systems and renders protection during inflammation.

TNF-α, a proinflammatory cytokine is a pivotal player in arthritis among several animal species, including mice and rabbit models. TNF-α is produced primarily by stimulated monocytes, macrophages and synovial lining cells (Divya Singh *et al*., 2006). Inflammation is usually marked by abundance of proinflammatory cytokines. This inflammatory mediator TNF-α is also responsible for the cartilage and bone damage. Our results indicated that *Spirulina fusiformis* suppressed the inflammatory process by reducing the production of tumour necrosis factor-α in monosodium urate crystal-induced mice.

The polymorphonuclear leucocyte cells are implicated in allergic and inflammatory responses due to their increased recruitment at the site of inflammation and secretion of elevated levels of cysteinyl leukotrienes, which culminate in pathophysiological conditions. Polymorphonuclear leucocytes is supposed to contain substances capable of mediating physiological changes characteristic of inflammation; such substances may be cytoplasmic or lysosomal (Weissmann, 1972). Monosodium urate crystals can cause enzyme release from polymorphonuclear leucocytes cells mainly by a direct effect on the plasma membrane. Interaction between crystals and cell membrane can also alter cellular metabolism, and cause secretion of lysosomal enzymes from leucocytes in the absence of phagocytosis (Wallingford and McCarty , 1971). In the present study, human polymorphonuclear leucocyte cells, significantly released α-glucuronidase and lactate dehydrogenase upon exposure to monosodium urate crystals. *Spirulina fusiformis* suppresses the monosodium urate crystal-induced enzyme release from polymorphonuclear leucocyte cells suggesting that it could prevent normal tissues from injurious effect of these enzymes and tend to retard inflammatory response.

From the present study, mechanism of action of *Spirulina fusiformis* in reducing the monosodium urate crystal-induced inflammation is not known. On the basis of our results we conclude that the *Spirulina fusiformis* may exert its anti-arthritic activity by retarding amplification and propagation of the inflammatory response. The observed anti-arthritic effect of *Spirulina fusiformis* might be due

to its components like â-carotene, Vitamin C, E, enzyme superoxide dismutase and selenium which has been described (Sowers and Lachance, 1999).

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