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Therapeutic Efficacy of *Spirulina* In the Treatment of Formaldehyde Induced Rheumatoid Arthritis in Swiss Albino Mice

B. Dheeba[1]*, P. Sampathkumar[1], M. Kannan[2], E.Vaishnavi[2] and Maragatham[1]

[1]Department of Chemistry and Biosciences, Srinivasa Ramanujan Centre, SASTRA University, Kumbakonam - 612 001, India .
[2]Department of Microbiology, V.H.N.S.N. College, Virudhunagar - 626 001, India.

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The present study evaluates the anti-arthritic effect of *Spirulina fusiformis* on formaldehyde induced Rheumatoid arthritis in mice. Arthritis was induced by intradermal injection of formaldehyde (0.1 ml of 2%v/v) into the right hind paw of Swiss albino mice. *Spirulina fusiformis* (500mg/kg b.wt & 1000mg/kg/b.wt) was orally administrated for 10 days. The anti arthritic effect of *Spirulina fusiformis* was evaluated by measuring changes in the paw volume, body weight, protein, uric acid, serum and liver lysosomal enzymes such as Acid phosphatase, β-glucuronidase and β- galactosidase, protein-bound carbohydrates such as hexose, hexosamine, fucose in control and experimental groups. In formaldehyde induced arthritic animals, the levels of lysosomal enzymes in serum and liver were decreased and the levels of protein-bound carbohydrates and paw volume were increased. However the body weight was found to be reduced when compared to control animals. Oral administration of *Spirulina fusiformis* (1000mg/kg/b.wt) significantly altered these physical and biochemical changes observed in arthritic animals to near normal conditions. Hence results of this study clearly indicate that *Spirulina fusiformis* has promising anti-arthritic activity against formaldehyde induced rheumatoid arthritic animals.

Key words: *Spirulina fusiformis*, Formaldehyde, Rheumatoid Arthritis, Anti-inflammatory.

Rheumatoid arthritis (RA) has been well characterized by symmetric polyarthritis affecting several joints, accompanying synovial hyperplasia, consequently leading to joint destruction and deformity, loss of function, and reduced quality of life. The prevalence of major rheumatic disease in adult range from 24% in China and Indonesia, but 45% in Philippines, Chile and rural Africa and affecting 0.5-1% of the entire human population[1].

Arthritis research, in general, has focused predominately on the metabolic turn over of collagen and cartilage degradation. However, full understanding of the molecular mechanism of cartilage damage requires more complex analysis of events, including evaluation of the role of lysosomal enzymes. Numerous studies have indicated that local release of lysosomal enzyme mediates atleast part acute and chronic inflammation in joints and in the synovial fluid in RA [2]. Currently steroids, non-steroidal anti-inflammatory drugs (NSAIDs) and immunosuppressant drugs are used in the relief of inflammation in RA and are often associated with severe adverse effect, the most common being gastrointestinal bleeding and peptic ulcers[3]. For this reason, more effective and safe drug with

* To whom all correspondence should be addressed.
Tel: +91 – 9443281398
E-mail: deepabaskaran76@gmail.com, deepabaskaran76@rediffmail.com

minimal side effects is required. Natural products are abundant source of pharmacologically active compounds, many of which have become important human drug [4].

Spirulina is a blue green algae of the Oscillateriaceae family which grows naturally in warm climate countries and has been considered as supplement in human and animal food[5]. It is safer for human conception has also been established through numerous toxicological studies[6] and gaining more attention for the treatment of wide spectrum of disease. It is known to have high protein contents, and natural biochelated vitamins. Some properties of *Spirulina* experimentally proved include antiviral, anticancer effect, strengthens immune system radioprotective and anti-inflammatory effects[7]. The present study was designed to demonstrate the novel evidence of therapeutic efficacy of *Spirulina fusiformis* on formaldehyde induced RA.

MATERIALS AND METHODS

Animals

Healthy Swiss albino mice weighed 25-30 g, of either sex were obtained from Tamilnadu Veterinary College, Chennai, India. The animals were acclimatized for a week in a light and temperature controlled room with a 12 h dark-light cycle and fed with commercial pelleted feed from Hindustan Lever Ltd. (Mumbai, India) and water *ad libitum*. The usage and handling of experimental mice was done followed by the rules and regulations given by the Institutional Ethics Committee.

Chemicals

The commercially available *Spirulina* (a fine-dark blue green spray-dried powder) was obtained from, Nallayan Research Centre, Nattam village, Kanjipuram Dt., Tamilnadu, India. All other reagents used were of highest purity and of analytical grade marketed by standard chemical companies.

Experimental design

The animals were divided into 4 groups of six animals each.

Group I : Normal control, received only the standard diet.

Group II: Experimental control. Arthritis was induced by intradermal injection of 0.1ml formaldehyde (2% v/v) solution through the right hind paw .

Group III : Mice were treated orally with *Spirulina fusiformis* (500 mg Kg^{-1}b.wt. day^{-1} for 10 days) in distilled water. At 11th day administrated 0.1 ml of formaldehyde (2% v/v) solution through the right hind paw.

Group IV : Mice were treated orally with *Spirulina fusiformis* (1000 mg Kg^{-1}b.wt. day^{-1} for 10 days) in distilled water. At 11th day administrated 0.1 ml of formaldehyde (2% v/v) solution through the right hind paw.

Collection of sample

On the 21st day, at the end of the experimental period, the animals were sacrificed by cervical decapitation and blood was collected and used for measurement of lysosomal enzymes and protein bound carbohydrates. The liver was immediately dissected out and homogenized in ice-cold 0.01 M Tris HCl buffer pH 7.4 to give a 10% homogenate used for assay. Arthritis was assessed by means of physical and biochemical measurements. Paw volume was assessed by measurement of the right hind paw by use of a vernier scale and body weight was recorded in both control and experimental animals.

Analytical method

Acid phosphatase[8] , β –Glucuronidase[9] and β- Galactosidase[10] were determined. Glycoprotein analysis, a known amount of liver tissue was defatted by dissolving in hexane and taken in test tube, to which 1 ml of 2 N HCl was added, and the tubes were sealed. Hydrolysis was complete by keeping the sealed tubes in 100°C for 16-18 h. After hydrolysis, the contents were neutralized with NaOH and made up to known volume, and aliquots were used for glycoproteins such as hexose[11], hexosamine[12], fucose[13] determination.

Statistical analysis

Results are expressed as mean ± S.D. One-way ANOVA was carried out and the statistical comparisons among the groups were performed by using a statistical software package program SPSS 7.5.

RESULTS

Table 1 shows the effect of *Spirulina fusiformis* on paw volume in control and experimental animals. Measurement of the paw volume of mice with formaldehyde- induced arthritis (Group II) revealed an increase in ankle

Table 1. Effects of *Spirulina fusiformis* on paw volume in formaldehyde -induced arthritic mice.

Groups	Paw volume (mm)					
	0	4th day	8th day	12th day	16th day	20th day
Group I	0.2±0.01	0.2±0.01	0.2±0.01	0.2±0.01	0.2±0.01	0.2±0.01
Group II	0.2±0.01	0.4±0.02	0.8±0.06	1.00±0.06	1.15±0.08	1.20±0.10^{a*}
Group III	0.2±0.01	0.2±0.01	0.2±0.01	0.23±0.01	0.26±0.01	0.3±0.02
Group IV	0.2±0.01	0.2±0.01	0.2±0.01	0.21±0.01	0.22±0.01	0.22±0.01^{b*c*}

Values are expressed as mean ±S.D of six animals in each group.
Statistical Significance at: * P<0.05. a* - control mice Vs arthritic mice; b* - arthritic mice Vs treated arthritic mice; c* - Group III mice Vs Group IV mice.

Table 2. Effect of *Spirulina fusiformis* on body weight changes

Groups	Body weight (grams)					
	0	4th day	8th day	12th day	16th day	20th day
Group I	28±0.81	27±0.79	28±0.82	28±0.82	29±0.80	30±0.90
Group II	28±0.81	25±2.00	20±1.64	17±0.52	16.5±0.50	15±0.01^{a*}
Group III	25±0.81	26±0582	27±0.0.65	28±0.82	27.5±0.65	27±0.65
Group IV	26±0.51	28±0.82	30±2.10	31±0.85	31±0.85	31±0.0.95^{b*c*}

Values are expressed as mean ±S.D of six animals in each group.
Statistical Significance at:* P<0.05. a* - control mice Vs arthritic mice; b* - arthritic mice Vs treated arthritic mice; c* - Group III mice Vs Group IV mice.

Table 3. Effect of *Spirulina fusiformis* on the activities of lysosomal enzymes in serum and liver

Groups	Serum			Liver		
	ACP	β-glucuronidase	β-galactosidase	ACP	β-glucuronidase	β-galactosidase
Group I	0.12±0.02	1.60±0.04	1.01±0.1	2.50±0.12	27.21±0.16	11.21±0.05
Group II	0.85±0.04^{a*}	6.01±1.02^{a*}	3.21±0.25^{a*}	3.63±0.41^{a*}	43.56±1.78^{a*}	20.76±0.21^{a*}
GroupIII	0.38±0.01	2.38±0.21	1.60±0.11	2.82±0.24	38.21±2.01	15.23±0.19
GroupIV	0.14±0.01^{b*c*}	1.72±0.81^{b*c*}	1.12±0.45^{b*c*}	2.56±0.12^{b*c*}	28.25±0.95^{b*c*}	11.75±0.24^{b*c*}

Values are expressed as mean ±S.D of six animals in each group. Statistical Significance at: *P<0.05.a*- control mice Vs arthritic mice. b* - arthritic mice Vs treated arthritic mice; c* - Group III mice Vs Group IV mice. Enzyme activities are expressed as: acid phosphatase: µmol of phenol liberated/min/mg protein; â-glucuronidase: µ moles x 10^{-2} DNP liberated/hr/ protein and â -galactosidase: µ moles x 10^{-2} ONP liberated/hr/ protein.

diameter from day 4, which increases further up to 20th day. The administration of *Spirulina fusiformis* to Group III and Group IV shows significant (P<0.05) effects, when compared to Group II. While comparing group III and group IV less paw volume shown in group IV animals which recieved 1000mg/ kg b.wt.

Table 2 shows the body weight changes of the control and experimental mice. The growth of arthritic mice (Group II) was found to be retarded. The body weight of *Spirulina fusiformis* treated animals (Group III and Group IV) were found to increase when compared to that of control animals. Especially in Group IV which was treated with

Table 4. Effect of *Spirulina fusiformis* on protein bound carbohydrates in liver

Groups	Hexosamine (mg/g tissue)	Hexose (mg/g tissue)	Fucose (mg/g tissue)
Group I	0.15±0.20	2.50±0.32	0.64±0.01
Group II	0.42±0.05^{a*}	4.0±0.94^{a*}	0.90±0.05^{a*}
Group III	0.18±0.23^{b*c*}	2.52±0.12^{b*c*}	0.65±0.03^{b*c*}
Group IV	0.21±0.10	2.83±0.41^{b*}	0.68±0.02^{b*}

Values are expressed as mean ±S.D of six animals in each group.
Statistical Significance at: *P<0.05.a*, control mice Vs arthritic mice; a*- control mice Vs arthritic mice; b* - arthritic mice Vs treated arthritic mice; c* - Group III mice Vs Group IV mice.

1000mg/kg body weight of *Spirulina,* significant increase (P<0.05) in body weight was noticed.

Table 3 depicts the activities of lysosomal enzymes in serum and liver of control and experimental animals. In formaldehyde-induced arthritic animals, (Group II) the activities of acid phosphatase, â-glucuronidase, â-galactosidase levels were increased when compared to control animals. The *Spirulina fusiformis* administered mice (Group III, Group IV) having better results than arthritic mice (Group II).

The effect of *Spirulina* on the glycoprotein levels in liver homogenate of control and experimental animals were given in table 4. The sugar componenets of glycoproteins- hexose, hexosamine and fucose were significantly increased (P<0.05) in arthritic animals (Group II) than control (Group I) animals. *Spirulina fusiformis* administrated arthritic mice (Group III, Group IV) had decreased level of glycoproteins significantly. When compared Group III and group IV, group IV shows better results, *Spirulina fusiformis* having both preventive as well as protective effects.

DISCUSSION

RA is a complicated refractory autoimmune disease characterized by a number of inflammatory and destructive events such as joint pain and swelling, synovial hyperplasia, pannus formation, cartilage and bone erosions, joint malformation *etc.*[14]. Treatment decisions in rheumatoid arthritis are individualized and depend largely on the disease activity at the time presentation. Many drugs are clinically used for the treatment of rheumatoid arthritis, but therapeutic effects are restricted by their side effects. For example, hormone can cause osteoporosis; methotrexate can inhibit bone marrow proliferation and cause leukopenia and thrombocytopenia; and leffunomide can lead to gastrointestinal dysfunction, skin rash, allergic reaction, loss of body weight, and reversible baldness[15].

Increased paw swelling (Table 1) observed in the arthritic mice was found to be a result of oedema of peri artricular tissues. An increase in granulocytes and monocytes has been found to be associated with changes in ankle diameter[16]. Paw swelling was significantly reduced in arthritic mice treated with *Spirulina fusiformis*, which indicates its interference on cyclo-oxgenase pathway. Changes in the body weight is in response to the incidence and severity of arthritis and used to assess the onset of the diseases. The weight loss (Table 2) is associated with increased production of pro-inflammatory cytokine such as tumour necrosis factor-á and IL_1 and an increased prevalence of low body mass in RA population. Treatment of *Spirulina fusiformis* recovered the body weight ,which further support the anti-arthritic effect[17].

Lysosomes are group of cytoplasmic organelles present in numerous animal tissue characterized by their content of acid hydrolase. Evidence in increasing lysosomal enzymes play an important role in inflammation. It is well known that immune complexes are endocytosed by leukocytes followed by the extraction of lysosomal hydrolases into the extracellular environment.

The involment of liver lysosomes in the process of endocytosis is also well documented[18]. Reduction of the release of lysosomal enzymes in both serum and liver tissues would prove beneficial and this indirectly confirms the protective effect of the drug due to the presence of some phenolic components and that have membrane stabilizing effects. *Spirulina fusiformis* administration decreases the lysosomal enzyme (Table 3) release and stabilizing effect in formaldehyde-induced rheumatoid arthritic mice, which indicates its anti-inflammatory effect.

Glycoproteins are the components of connective tissue that are responsible for the antigenic property in tissue transplants and maintaining the structural stability of collagen fibril[19]. The altered glycoprotein metabolism observation in arthritic animals (Group II) is due to the increased release of acid hydrolase during arthritic condition. Those enzymes are involved in the degradation of structural macromolecules in connective tissue and cartilage proteoglycans. The metabolic turnover was found to be increased in the ligament and cartilage during the inflammatory process of arthritis[20]. After *Spirulina fusiformis* treatment (Group III and IV) glycoprotein levels were significantly decreased which may be due to its modulating role on lysosomal hydrolases.

There is an increasing interest in herbal medications especially for chronic diseases like RA[21] and plant remedies have become increasingly popular and are often preferred to synthetically derived pharmaceuticals. It is therefore of interest to determine their active components and to elucidate their molecular mechanisms of action[22]. The present study concludes that *Spirulina fusiformis* is able to suppress the changes produced during formaldehyde-induced arthritis.

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