

EVALUATION OF THE PROTECTIVE POTENTIAL OF *SPIRULINA FUSIFORMIS* ON LIPID PEROXIDATION AND ANTIOXIDANT STATUS IN ADJUVANT-INDUCED ARTHRITIC MICE

M. Rasool^{1*} and E.P. Sabina,²

¹Faculty of Health and Life Sciences, Management and Science University, Shah Alam, Malaysia, ²School of Biotechnology, Chemical and Biomedical engineering, VIT University Vellore – 632 014, Tamil Nadu, India.

Summary

The aim of this research is to determine the anti-peroxidative potential of *Spirulina fusiformis* in adjuvant-induced arthritis with respect to lipid peroxidation, and antioxidant status. Arthritis was induced by intradermal injection of Complete Freund's Adjuvant (0.1 ml) into the right hind paw of Swiss albino mice. *Spirulina fusiformis* (800mg/kg/day) and Indomethacin (3mg/kg/day) were orally administered for 8 days (from 11th to 18th day) after adjuvant injection. The lipid peroxide level, enzymic and non-enzymic-antioxidants status were measured in blood, liver and spleen of control and arthritic animals. In arthritic animals, the lipid peroxidation was found to be increased, whereas the decreased activities of antioxidant enzymes superoxide dismutase, catalase and glutathione peroxidase and non-enzymic antioxidant levels (Glutathione, vitamin C, and thiol groups), were observed. These observed alterations were significantly modulated to near normal levels by oral administration of *Spirulina fusiformis* (800mg/kg/day) in arthritic animals. In conclusion, the present study provides evidence that it may have a promising anti-arthritic role against rheumatoid arthritis. However, biochemical and metabolic analysis of the constituents of *Spirulina fusiformis* is on underway to investigate its mechanism of action.

Keywords: *Spirulina fusiformis*, adjuvant-induced arthritis, lipid peroxidation, antioxidants

*Corresponding author:

Dr. M. Rasool M. Sc., Ph.D.
Faculty of Health and Life Sciences,
Management and Science University,
40100 Shah Alam, Selangor Darul-Ehsan
Malaysia
E-mail: mkr474@gmail.com

Introduction

Rheumatoid arthritis (RA) is the most common form of inflammatory arthritis, a systemic autoimmune disease that primarily presents as a chronic symmetric polyarthritis associated with inflammation and cartilage destruction, affecting 1% of the world population (1). Despite extensive research, the pathogenesis of rheumatoid arthritis remains incompletely understood. However, in recent years increasing experimental and clinical data have provided evidences for the involvement of free radicals in rheumatoid arthritis (2). Free radicals are formed during normal aerobic metabolism in all cells (e.g. via leakage from the mitochondrial electron transport chain) followed by phagocyte activation during infection/inflammation. Due to the process of evolution, mammalian cells have developed antioxidant defense systems like endogenous free radical scavenging proteins, enzymes and chemical compounds for survival in an aerobic environment (3). The failure of antioxidant defence system may either due to decrease in antioxidant defense or increased generation of oxidants. Although a number of drugs (non-steroidal or steroidal anti-inflammatory agents and immunosuppressant) used in the treatment of rheumatoid arthritis have been developed over the past few decades, there is still an urgent need for a more effective drug capable of preventing disease progression or reverting joint destruction.

Spirulina is blue green algae of the Oscillatoriaceae family which grows naturally in countries which have a warm climate and has been considered as supplement in human and animal food (4). They have been found to be a rich source of vitamins, minerals, essential fatty acids and antioxidant pigments such as carotenoids (5).

In addition, several studies have shown that *Spirulina* species exhibit various biological activities such as antitumour (6), antimicrobial (7), strengthening immune system (8), radio protective (9), and metalloprotective effect (10).

Our preliminary studies confirm its anti-inflammatory and lysosomal membrane stabilizing effect on adjuvant-induced arthritis in mice (4). In the current study, the anti-arthritic effect of *Spirulina fusiformis* was investigated using adjuvant-induced arthritis, a well known experimental model for rheumatoid arthritis in mice. The anti-arthritic effect of *Spirulina fusiformis* was assessed by measuring changes in lipid peroxidation, and antioxidant status in plasma, liver and spleen of arthritic animals. The standard non-steroidal anti-inflammatory drug, Indomethacin, was used as a reference drug for purposes of comparison.

Materials and methods

Animals

The study was performed with Swiss albino mice, 25-30g, of either sex. The mice were brought from the Tamil Nadu Veterinary College, Chennai, India. The mice were acclimatized for a week in a light and temperature –controlled room with a 12 hr dark-light cycle. The mice were fed commercial pelleted feed from Hindustan Lever Ltd. (Mumbai, India) and water was made freely available.

Drug

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 aqueous suspension. This aqueous suspension of *Spirulina fusiformis* was used as an oral dose of 800 mg/kg/b.wt, orally. All other reagents used were standard laboratory reagents of analytical grade which were purchased locally.

Dosage

Based on our preliminary studies with different dosages (200mg, 400mg, 600mg, 800mg) of this drug, it was found that 800mg/kg b.w dosage produced significant anti-inflammatory effect by reducing paw swelling in adjuvant – induced arthritic animals. Hence 800mg/kg b.w dosage was considered for this study.

Experimental protocol

The mice were divided into six groups each comprising six animals. Group 1 served as controls. In Group II, arthritis was induced by intra dermal injection of Complete Freund's Adjuvant (CFA) (0.1ml) into the right hind paw (11). The adjuvant (Tuberculosis Research Center, Chennai, India) contained heat-killed *Mycobacterium tuberculosis* (H37 RA) (10mg) in paraffin oil (1ml). Group III and IV were treated with *Spirulina fusiformis* and Indomethacin, respectively, for 8 days from the 11th to the 18th day of the experimental period. Groups V and VI comprised of arthritic mice were treated with *Spirulina fusiformis* and Indomethacin, respectively, from day 11th to 18th after the administration of Complete Freund's Adjuvant (CFA).

On the 19th day, at the end of the experimental period, the animals were sacrificed by cervical decapitation and the blood was collected. 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. Tissue homogenates and plasma were used for assaying the following biochemical investigations.

Biochemical estimations

Lipid peroxidation in plasma was estimated by the method of Ledwozy et al (12). Liver and spleen lipid peroxidation were carried out by the procedure of Hogberg et al (13) using thiobarbutric acid (TBA) as the colouring agent. Malonaldehyde (MDA) produced during peroxidation of lipids served as an index of lipid peroxidation. Malonaldehyde reacts with thiobarbutric acid to generate a colour product, which absorbs at 532 nm.

Liver and spleen lipid peroxidation with inducer system namely, 10mM FeSo₄ / 0.2 ascorbate /10mM H₂O₂ was measured by the method of Devasagayam (14). The Malonaldehyde contents of the samples were expressed as nmoles of MDA formed/mg/protein.

Superoxide dismutase (SOD) activity in liver and spleen was determined by the method of Marklund and Marklund (15). The degree of inhibition of the auto-oxidation of pyrogallol at an alkaline pH by SOD was used as a measure of the enzyme activity. Catalase and glutathione peroxidase activities in liver and spleen were estimated by the method of Sinha (16) and Rotruck et al (17). The activity of catalase was expressed as µg of H₂O₂ consumed/mn/mg protein. Glutathione peroxidase was expressed as µg of glutathione utilized /minute/mg/ protein. Non enzymic antioxidants, glutathione (18), ascorbic acid (19), total sulphhydryl (TSH) and non protein sulphhydryl (NPSH) groups (20) were estimated in the liver and spleen.

Statistical analysis

Results were expressed as mean ± S.D. and a statistical analysis was performed using ANOVA to determine significant differences between groups, followed by Student's Newman-Keul's test. P< 0.05 implied significance.

Results

Table 1 represents the levels of lipid peroxide in plasma, liver, and spleen of control and arthritic animals. In group II arthritic mice, basal lipid peroxide levels were elevated in plasma, liver and spleen. In presence of inducers like ascorbate, feso₄, and H₂O₂, liver and spleen lipid peroxide levels were found to be increased significantly compared to control group, whereas the administration of *Spirulina fusiformis* to arthritic mice altered the above changes by regulating the lipid peroxide level to nearly that of normal levels.

Table 2 and 3 depicts the activities of enzymic (SOD, GPx and Catalase) and levels of non-enzymic antioxidants in spleen and liver of control and experimental animals respectively. The enzymic and non-enzymic antioxidants were significantly decreased in group II arthritic mice when compared to control mice. These changes highlight the deteriorating antioxidant status in the arthritic mice. Furthermore, administration of *Spirulina fusiformis* increased the both spleen and liver enzymic and non-enzymic antioxidant levels in arthritic mice considerably, which indicates its antiperoxidative action.

Table 1. Effect of *Spirulina fusiformis* and Indomethacin on basal and induced lipid peroxidation in control and experimental animals

Parameter	Group I Control	Group II Arthritis	Group III <i>Spirulina fusiformis</i>	Group IV Indomethacin	Group V Arthritis + <i>Spirulina fusiformis</i>	Group VI Arthritis+ Indomethacin
Plasma	2.75 ± 0.18	5.45± 0.34 a*	2.65± 0.14	2.72± 0.34	2.85± 0.15 a*b*	3.20± 0.20a*b*
Spleen						
Basal	2.65 ± 0.16	5.75± 0.44 a*	2.50± 0.10	2.62± 0.18	2.95± 0.20 a*b*	3.75± 0.22 a*b* c*
FeSO ₄ Induced	3.1 ± 0.23	5.50± 0.32 a*	3.15± 0.16	3.16 ± 0.18	3.75 ± 0.26 a*b*	3.50± 0.21 a*b*
Ascorbate Induced	3.25 ± 0.20	6.50± 0.40 a*	3.05± 0.15	3.26± 0.19	3.82± 0.28 a*b*	4.35± 0.25 a*b* c*
H ₂ O ₂ Induced	2.45 ± 0.18	4.1± 0.22 a*	2.40± 0.10	2.50± 0.13	3.15± 0.18 a*b*	3.1± 0.17 a*b*
Liver						
Basal	1.85± 0.15	3.13± 0.03a*	1.92± 0.20	1.90± 0.15	1.96 ± 0.17 a*b*	2.50± 0.22 a*b*c*
FeSO ₄ induced	5.06± 0.52	6.57± 0.23 a*	5.02± 0.53	5.12± 0.44	5.75 ± 0.55 a* b*	5.45 ± 0.65 a* b*
Ascorbate induced	4.58± 0.45	6.85± 0.56 a*	4.85± 0.42	4.64± 0.30	4.95± 0.41 a* b*	5.70± 0.56 a*b* c*
H ₂ O ₂ induced	2.30± 0.20	3.61 ± 0.15 a*	2.50± 0.20	2.55 ± 0.22	2.50± 0.21 a* b*	2.78± 0.31 a*b*

Treatment of groups are as follows : Group I-Control; Group II-Arthritic mice; Group III-Control mice treated with *Spirulina fusiformis* (800mg/kg b.wt) for 8 days from 11th to 18th day ; Group IV- Control mice treated with Indomethacin (3mg/kg b.wt) for 8 days from 11th to 18th day ; Group V –Arthritic mice treated with *Spirulina fusiformis* (800mg/kg b.wt) from 11th to 18th day post adjuvant; Group VI- Arthritic mice treated with Indomethacin (3mg/kg b.wt) from 11th to 18th day post adjuvant. **Comparisons are made as follows:** a – Group I vs groups II, III, IV, V, and VI; b - Group II vs group V, and VI

Each value represent mean± SD (n=6). Values are expressed as: nanomoles of malonaldehyde formed /mg protein; plasma-mg/dl. The symbols represent statistical significance at: * p < 0.05.

Table 2. Effect of *Spirulina fusiformis* and Indomethacin on the enzymic and nonenzymic antioxidant levels in spleen of control and experimental animals

Parameter	Group I Control	Group II Arthritis	Group III <i>Spirulina fusiformis</i>	Group IV Indomethacin	Group V Arthritis + <i>Spirulina fusiformis</i>	Group VI Arthritis+ Indomethacin
Superoxide Dismutase (SOD)	1.50 ± 0.10	0.90 ± 0.05 a*	1.55 ± 0.08	1.55 ± 0.12	1.35 ± 0.15 b*	1.60 ± 0.12 a*b*
Glutathione peroxidase (Gpx)	3.22 ± 0.18	2.54 ± 0.16 a*	3.25 ± 0.15	3.25 ± 0.20	3.01 ± 0.14 a*b*	3.19 ± 0.19 a* b*
Catalase (CAT)	15.50 ± 1.29	10.50 ± 0.80 a*	16.50 ± 1.78	15.80 ± 1.05	13.05 ± 0.72 a*b*	13.70 ± 1.05 a*b*
Glutathione Total sulphhydryl (TSH)	1.76 ± 0.05	1.23 ± 0.08 a*	1.95 ± 0.10	1.70 ± 0.05	1.50 ± 0.10 a* b*	1.65 ± 0.10 a*b*
Non-protein sulphhydryl (NPSH)	7.50 ± 0.46	4.36 ± 0.27 a*	7.60 ± 0.55	7.65 ± 0.58	6.40 ± 0.50 a* b*	7.10 ± 0.47 a* b*
Vitamin C	1.85 ± 0.14	1.35 ± 0.07 a*	1.80 ± 1.01	1.82 ± 0.16	1.60 ± 0.01 b*	1.73 ± 0.10 a*b*
	2.05 ± 0.12	1.25 ± 0.07 a*	2.10 ± 0.18	1.90 ± 0.15	1.70 ± 0.09 a*b*	1.88 ± 0.13 a*b*

Treatment of groups are as follows : Group I-Control; Group II-Arthritic mice; Group III-Control mice treated with *Spirulina fusiformis* (800mg/kg b.wt) for 8 days from 11th to 18th day ; Group IV- Control mice treated with Indomethacin (3mg/kg b.wt) for 8 days from 11th to 18th day ; Group V –Arthritic mice treated with *Spirulina fusiformis* (800mg/kg b.wt) from 11th to 18th day; Group VI- Arthritic mice treated with Indomethacin (3mg/kg b.wt) from 11th to 18th day.

Comparisons are made as follows: a – Group I vs groups II, III, IV, V, and VI
b - Group II vs group V, and VI

Values are expressed as mean ± SD (n=6). Enzyme units are expressed as; SOD-units/mg protein (unit-Amount of enzyme required to inhibit the auto-oxidation reaction by 50 %); Gpx-µg of GSH utilized / min/mg protein; Catalase- µmol of H₂O₂ consumed /min/mg protein. Glutathione, Total sulphhydryl (TSH), Non-protein sulphhydryl (NPSH), Vitamin C- µg/mg protein. The symbols represent statistical significance at: * p < 0.05.

Table 3. Effect of *Spirulina fusiformis* and Indomethacin on the enzymic and nonenzymic antioxidant levels in liver of control and experimental animals

Parameter	Group I Control	Group II Arthritis	Group III <i>Spirulina fusiformis</i>	Group IV Indomethacin	Group V Arthritis + <i>Spirulina fusiformis</i>	Group VI Arthritis+ Indomethacin
Superoxide Dismutase (SOD)	2.10 ± 0.10	1.20 ± 0.05 a*	1.95 ± 0.10	2.15 ± 0.12	1.80 ± 0.11 b*	1.65 ± 0.12 a*b*
Glutathione peroxidase(Gpx)	5.22 ± 0.18	3.54 ± 0.16 a*	5.35 ± 0.38	5.25 ± 0.20	4.75 ± 0.24 a*b*	4.39 ± 0.22 a* b*
Catalase (CAT)	5.50 ± 0.29	3.75 ± 0.40 a*	5.70 ± 0.31	5.80 ± 1.05	4.90 ± 0.24 a*b*	4.70 ± 1.05 a*b*
Glutathione Total	5.76 ± 0.25	2.35 ± 0.05 a*	5.80 ± 0.32	5.70 ± 0.35	4.85 ± 0.21 a* b*	4.65 ± 0.10 a*b*
sulphydryl (TSH)	6.50 ± 0.46	3.56 ± 0.25 a*	6.75 ± 0.53	6.65 ± 0.41	5.2 ± 0.36 a* b*	5.10 ± 0.35 a* b*
Non-protein sulphydryl (NPSH)	5.85 ± 0.24	3.35 ± 0.25 a*	5.95 ± 0.59	5.82 ± 0.36	4.85 ± 0.21 b*	4.73 ± 0.20 a*b*
Vitamin C	2.55 ± 0.12	1.20 ± 0.05 a*	2.65 ± 0.23	2.70 ± 0.15	2.15 ± 0.11 a*b*	2.25 ± 0.12 a*b*

Treatment of groups are as follows : Group I-Control; Group II-Arthritic mice; Group III-Control mice treated with *Spirulina fusiformis* (800mg/kg b.wt) for 8 days from 11th to 18th day ; Group IV- Control mice treated with Indomethacin (3mg/kg b.wt) for 8 days from 11th to 18th day ; Group V –Arthritic mice treated with *Spirulina fusiformis* (800mg/kg b.wt) from 11th to 18th day; Group VI- Arthritic mice treated with Indomethacin (3mg/kg b.wt) from 11th to 18th day.

Comparisons are made as follows: a – Group I vs groups II, III, IV, V, and VI

b - Group II vs group V, and VI

Values are expressed as mean ± SD (n=6). Enzyme units are expressed as; SOD-units/mg protein (unit-Amount of enzyme required to inhibit the auto-oxidation reaction by 50 %); Gpx-µg of GSH utilized / min/mg protein; Catalase- µmol of H₂O₂ consumed /min/mg protein. Glutathione, Total sulphydryl (TSH), Non-protein sulphydryl (NPSH), Vitamin C- µg/mg protein. The symbols represent statistical significance at: * p < 0.05.

Discussion

Rheumatoid arthritis is a debilitating autoimmune disease and has a substantial social effect in terms of cost, disability and lost productivity. Although the precise mechanisms responsible for the differential pathogenesis of rheumatoid arthritis remain unknown, some investigators have focused on oxidative stress in rheumatoid inflammation. A free radical is any atom, molecule, or ion that contains one or more unpaired electrons that might be formed during a number of physiologic or pathologic reactions. These unmapped electrons can initiate destructive reactions against proteins or lipids and nucleic acid in the body, which reactivates the free radicals and increases the essence of these radicals in pathology of many diseases including rheumatoid arthritis. In the previous studies, we found that *Spirulina fusiformis* effectively suppressed the release of lysosomal acid hydrolases in adjuvant-induced arthritic mice (4). Here in the present study, we have evaluated the effect of *Spirulina fusiformis* on lipid peroxidation and antioxidant status in adjuvant-induced arthritis in mice; an experimental model of arthritis, which resembles several aspects of human rheumatoid arthritis (21).

In our study, the increased lipid peroxide observed in arthritic animals may be due to its release from neutrophils and monocytes during inflammation. It is an accepted fact that rheumatoid arthritis is accompanied by abnormalities in body iron metabolism. At the onset of inflammation, it was observed that there was a rapid fall in the 'total iron' content of blood plasma followed by an increased deposition of iron proteins in the synovial fluid. The drop in plasma iron correlates closely with the activity of the inflammatory process (22). In the synovial fluid of inflamed joints, the iron released during necrosis, might catalyze the formation of hydroxyl radicals from H_2O_2 , thus contributing to an increase in inflammation. It was observed that *Spirulina fusiformis* administration significantly decline lipid peroxide content in adjuvant-induced arthritic animals. The augmented lipid peroxidation observed in adjuvant arthritic animals might be compromised and counterbalanced by the several active components like β -carotene (5), vitamin C and E (23), enzyme super oxide dismutase (24), selenium (25) and brilliant blue polypeptide pigment phycocyanin (10) present in *Spirulina fusiformis*.

In our present study, the concentration of enzymic and non-enzymic antioxidants was significantly decreased in arthritic mice (Group II), as compared to normal control mice. Researchers namely as Heliovaara et al (26), Geetha et al (27), and Ram prasath et al (28) have suggested that enzymatic and/or nonenzymatic antioxidant systems are impaired in rheumatoid arthritis. Super oxide dismutase, zinc and copper-containing metalloenzyme, is a primary defense element against oxygen-derived free radicals, which catalyzes the dismutation of the superoxide anion into hydrogen peroxide. Then hydrogen peroxide can be transformed to H_2O and O_2 through catalase. The lowered levels of SOD activity observed in arthritic mice may be due to the inhibition of the enzyme by hydrogen peroxide, which might be an indicator of high degree of superoxide anion production. The increased rate of free radical production frequently results in a decrease in the level of antioxidant enzymes and the enzyme activities are reduced thereby leading to autocatalysis of oxidative damage process. Glutathione peroxidase is located in the cytoplasm and mitochondria of cells. It catalyzes the degradation of various peroxides by oxidizing glutathione with formation of its conjugates (29). Catalase is an another enzyme that functions together with super oxide dismutase and glutathione peroxidase through enzymic antioxidant defense system and catalyses the decomposition of hydrogen peroxide to water and oxygen to protect cells against O_2 toxicity and lipid peroxidation (28).

In our study, glutathione peroxidase and catalase were found to be reduced in arthritic mice, similar supporting evidences of low glutathione peroxidase and catalase activity in arthritic animals were reported by Vijayalakshmi et al (30), and Shah and Vohra (31). Due to increased lipid peroxidation in arthritic mice, the levels of free radicals overcome the saturation level. The high concentrations of free radicals inhibit the activity of antioxidants and hence the activities of these enzymes appear to be reduced in arthritic animals. Enzymic antioxidants are inactivated by the free radicals and hence the presence of non-enzymic antioxidants is presumably essential for the removal of these radicals. Vitamin C, glutathione and other thiols are water soluble antioxidants that remove free radical from cytosol by reacting directly with them (32). In the present observation, non-enzymic antioxidants were found to be significantly decreased in arthritic animals. The observed non-enzymic antioxidants depression in adjuvant-induced arthritis is associated with the protracted inflammatory phase of the disease. Glutathione acts as a substrate for glutathione peroxidase and glutathione-s-transferase during the breakdown of H₂O₂ and lipid peroxides (33). The concentration of glutathione is regulated through glutathione synthetase, glutathione peroxidase and glutathione reductase (34). Thus, the depletion of glutathione observed in group II arthritic mice will render the enzyme, glutathione peroxidase inactive and it is evident from the decreased activity of glutathione peroxidase in arthritic animals. Depletion of -SH groups observed in arthritic animals might be due to the glutathione depression and also due to oxidation of protein thiols (35). Vitamin C was significantly reduced in arthritic mice. This reduction was due to increased oxidative stress, which leads to damage of the membranes of various cell organelles.

In our study, it was observed that *Spirulina fusiformis* protects the arthritic animal from lipid peroxidation and increases the enzyme activities of superoxide dismutase, glutathione peroxidase, catalase, and non-enzymic antioxidant levels, which suggests that *Spirulina fusiformis* prevents the oxidative damage due to its preventive and protective effect. The antiperoxidative role of *Spirulina fusiformis* observed in this study may be attributed due to the presence of natural antioxidants like β -carotene, vitamin C and E, enzyme superoxide dismutase, selenium, and blue pigment phycocyanin (10). Most of these compounds have been reported to be a potent inhibitor of lipid peroxide formation, a scavenger of hydroxyl and superoxide radicals, and to increase the antioxidant enzyme (36), (37), (38), (39). Thus the modifying role of *Spirulina fusiformis* observed in our study may be due to the antiperoxidative action of its components that was reported earlier.

To conclude, by considering its antiperoxidative potential, *Spirulina fusiformis* has been regarded to be useful for the treatment of rheumatoid arthritis. However, biochemical and metabolic analysis of the constituents of *Spirulina fusiformis* is on underway to investigate its mechanism of action.

References

1. Costello JC, Halverson PB. A new era in rheumatoid arthritis treatment. Wisc. Med. J 2003;102 (7): 29-33.
2. Kamanli A, Naziroglu M, Aydilek N, et.al. Plasma lipid peroxidation and antioxidant levels in patient with rheumatoid arthritis. Cell Biochem Funct. 2004; 22: 53-57.
3. Karıncaoglu Y, Batcioglu K, Erdem T et al. The levels of plasma and salivary antioxidants in the patient with recurrent aphthous stomatitis. J Oral Pathol & Med. 2005; 34: 7-12.
4. Rasool M, Sabina EP, Lavanya B. Anti-inflammatory effect of *Spirulina fusiformis* on adjuvant-induced arthritis in mice. Biological & Pharmaceutical Bulletin 2006; 29(1): 2483-7.

5. Seshadri CV, Umesh BV, Manoharan R. β -carotene studies in *Spirulina* Bioresource Technology 1991; 38: 111-113.
6. Mittal A, Suresh Kumar PV, Banerjee S, Rao et al. Modulatory potential of *Spirulina fusiformis* on carcinogen metabolizing enzymes in Swiss albino mice. Phytotherapy Research 1999;13: 111-114.
7. Hayashi K, Hayashi T, Kojima I. A natural sulphated polysaccharide, calcium spirulin isolated from *Spirulina plantensis*: *in vitro* and *ex vivo* evaluation of anti herpes simplex virus and antihuman immunodeficiency virus activities. AIDS Research and Human Retroviruses 1996;12 (15);1463-1471.
8. Qureshi MA, Garlich JD, Kidd MT. Dietary *Spirulina plantensis* enhances humoral and cell mediated immune functions in chickens. Immunopharmacology and Immunotoxicology 1996;18: 465-476.
9. Verma S : Chemical modification of radiation response in Swiss albino mice. Ph.D. Thesis, University of Rajasthan, Jaipur (India). 2000.
10. Sharma MK, Sharma A, Kumar A, et.al. *Spirulina fusiformis* provides protection against mercury chloride induced oxidative stress in Swiss albino mice. Food and Chemical Toxicology 2007; 45:2412-2419.
11. Rasool M, Sabina EP. Anti-inflammatory effect of Indian ayurvedic herbal formulation Triphala on adjuvant-induced arthritic mice. Phytotherapy Research 2007; 21(9):889-94
12. Ledwozyw A, Michalak J, Stepień A, et al. The relationship between plasma triglycerides, cholesterol, total lipids and lipid peroxidation products during human atherosclerosis . Clin.Chim.Acta 1986; 155: 275-284.
13. Hogberg JRE, Larson A, Kristoferson, et al. NADPH-dependent reductase solubilised from microsomes of peroxidation and its activity. Biochem.Biophys.Res.Commun 1974; 56: 836-842.
14. Devasagayam TAP, Tarachand U. Decreased lipid peroxidation in rat kidney during gastation. Biochem.Biophys.Res.Commun 1987; 145: 134-138.
15. Marklund SL, Marklund G. Involvement of superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur. J. Biochem 1974; 47: 469-474.
16. Sinha AK, Colorimetric assay of Catalase. Anal.Biochem 1972; 47: 389-394.
17. Rotruck JT, Pope AL, Ganther HE, et al. Selenium, biochemical role as a component of glutathione peroxidase purification and assay. Science 1973;179: 588-590.
18. Moron MS, Depierre JW, Mannervik B. Levels of glutathione, glutathione reductase and glutathione-S-transferase activities in rat lung and liver. Biochim. Biophys. Acta 1979; 582: 67-78.
19. Omaye ST, Turnbull JD, Sauberlich HE. Selected methods for the determination of ascorbic acid in animal cell, tissues and fluids. Meth.Enzymol 1979;62: 1-11.
20. Sedlack J, Lindsay RH. Estimation of total protein bound and non-protein bound sulphhydryl groups in the tissue with ellman's reagent. Anal.Biochem 1968; 25:192-205.
21. Pearson CM, Waksman BH, Sharp JT. Studies of arthritis and other lesions induced in rats by injections of microbacterial adjuvants. J. Exp.Med 1964;113:485-509.
22. Gutteridge JMC. Antioxidant properties of the prtotein ceruloplasmin, albumin and transferrin. A study of the activity in serum and synovial fluid from patients with rheumatoid arthritis. Biochem.Biophys.Acta 1986; 869: 119-27.
23. Mathew B, Sankaranarayanan R, Nair PP, et al. Evaluation to chemoprevention of oral cancer with *Spirulina fusiformis*. Nutrition and Cancer 1995;24:194-202.

24. Ben-Amotz. Presentation of Polysaccharides from Micro-Algae Workshop. Duke University. 1997.
25. Henrikson R. Earth food Spirulina. Recolina Ltd. Ronore enterprises Inc., Launa beach, CA, 1989; 27-65.
26. Heliovara M, Knekt P, Aho K et al. Serum antioxidant and risk of rheumatoid arthritis. *Ann Rheum Dis* 1994; 53:51-53.
27. Geetha T, Varalakshmi P, Marylatha R. Effect of triterpenes from *Crateva nurvala* stem bark on lipid peroxidation in adjuvant induced arthritis in rats. *Pharmacological Res* 1998;37:191-195.
28. VanuRamkum Ramprasad, Palanivelu Shanthi, Panchanatham Sachdanandam. Evaluation of antioxidant effect of Semecarpus anacardium Linn.nut extract on the components of immune system in adjuvants arthritis. *Vascular Pharmacol* 2005; 42:179-186.
29. Islamov BI, Balabanova RM, Funtikov VA. Effect of bioresonance therapy on antioxidant system in lymphocytes in patients with rheumatoid arthritis. *Bull Exp Biol Med.* 2002;134: 248-250.
30. Vijayalakshmi T, Muthulakshmi V, Sachdanandam P. Effect of the milk extract of Semecarpus anacardium nut on adjuvant arthritis-a dose dependent study in Wistar albino rats. *Gen. Pharmacol* 1996; 27:1223-1226.
31. Shah ZA, Vohora SB. Antioxidant/restorative effects of calcined gold preparations used in Indian systems of medicine against global and focal models of ischaemia. *Pharm & Toxicol.* 2002; 90:254-259.
32. Allen RG. Oxygen-reactive species and antioxidant responses during development: the metabolic paradox by cellular differentiation. *Proceedings of the Society of Experimental Biology and Medicine* 1991; 196: 117-129.
33. Gambhir JK, Lali P, Jain AK. Correlation between blood antioxidant levels and lipid peroxidation in rheumatoid arthritis. *Clin.Biochem* 1997; 30: 351-355.
34. Braven J, Ansari N, Figgitt DP et al. A comparison of glutathione reductase and glutathione peroxidase activities in patients with rheumatoid arthritis and healthy adults. *Br J Rheumatol* 1989; 8:212-215.
35. Beck FJ, White house MW. Effect of adjuvant arthritis disease in rat in cyclophosphamide an isophosphamide metabolism. *Biochem Pharmacol* 1993;22:2453-2458.
36. Gerster H. Anticarcinogenic effect of common carotenoids. *International Journal of Vitamin and Nutrition Research* 1993; 63: 93-121.
37. Rao MV, Sharma PSN. Protective effect of Vitamin E against mercuric chloride reproductive toxicity in male mice. *Reproductive Toxicology* 2001;15 (16): 705-712.
38. Upasani CD, Khera A, Balaraman R. Effect of lead with vitamin E, C or Spirulina on malondialdehyde, conjugated dienes and hydroperoxides in rats. *Indian Journal of Experimental Biology* 2001;39: 70-74.
39. Bhat VB, Madyastha KM. C-phycoyanin: a potent peroxy radical scavenger *in vivo* and *in vitro*. *Biochemical and Biophysical Research Communications* 2000; 275 (1): 20-25.