INFLUENCE OF SPIRULINA FUSIFORMIS ON THE SODIUM VALPROATE INDUCED HEPATOTOXICITY AND OXIDATIVE STRESS.

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Summary

Spirulina is gaining more attention from medical scientists as a neutraceutical and pharmaceutical. Sodium valproate (SV) induced hepatotoxicity is the result of the increased generation of reactive oxygen metabolites and lipid peroxides in liver. The purpose of this study was to evaluate the role of spirulina on sodium valproate induced hepatotoxicity and oxidative stress in rats. Sodium valproate 18 mg/kg/po was administered by gavage for 60 days to induce hepatotoxicity in rats. Spirulina was suspended in 1% tween 80 at a dose of 1000 mg/kg was administered by gavage along with sodium valproate. The acitivity of serum enzymes (alanine transaminase, aspartate transaminase), bilirubin, oxidative stress parameters (lipid peroxidation), antioxidative defence markers (total antioxidant status) were evaluated in blood. Sodium valproate produced significant increase in the levels of AST, ALT, bilirubin, lipid peroxidation products and decreased total antioxidant levels. These changes were effectively reversed by spirulina. Spirulina fusiformis possess hepatoprotective activity by its ability to ameliorate lipid peroxidation through the free radical scavenging activity, which enhanced the levels of antioxidant defence system.

Key words spirulina, sodium valproate, hepatotoxicity, lipid peroxidation, total antioxidant status.

Introduction

Many xenobiotics (drugs and environmental chemicals) are capable of causing some degree of liver injury. The liver is prone to xenobiotic - induced injury because of its central role in xenobiotic metabolism, its portal location within the circulation and its anatomic and physiologic structure (1).

Sodium valproate is extensively used due to its efficacy against a wide range of seizure disorders. Sodium valproate is commonly prescribed for petitmal and focal epilepsy, migraine, neuropathic pain and bipolar disorder. It is well tolerated at therapeutic doses, the most common adverse effects are nausea, vomitting, anorexia, amenorrhoea, sedation, tremor, weight gain, alopecia and hepatic toxicity, hyperammonemic encephalopathy, coagulation disorders, and pancreatitis (2), severe hepatotoxicity resulting in death (3). Hepatotoxicity includes hepatocellular damage including microvesicular steatosis, cellular ballooning, intrahepatic cholestasis, proliferation of bile ducts and hepatic necrosis. Valproic acid is an established cause of microvesicular steatosis in young children. The lesion is accompanied in severe cases by inflammation, necrosis and bile duct injury (4). Alterations in mental status develop in patients receiving sodium valproate through direct hepatotoxic effect and its metabolites (5). Drug hepatotoxicity involves dynamic processes like toxic metabolite generation and its accumulation in the liver (6). Sodium valproate and its unsaturated metabolites, 2-ene VPA and (E)-2, (Z)-3'-diene VPA, produce dose-dependent cytotoxicity in primary cultures of rat hepatocytes (7). and induces functional changes in the liver and by the generation of free radical induced oxidative stress (8, 9, 10, 11).

Spirulina fusiformis is a blue green algae (mycobacterium) belonging to the family Oscillatoriaceae, has been consumed by man since ancient times. Spirulina is unique among blue-green algae because it has long history of safe use. In Mexico and central Africa it is used as a primary food source and is currently grown at large scale in many countries for commercial purpose as a nutritional supplement for its high proteins, vitamins and mineral contents (12). Spirulina`s dark color comes from a rainbow of natural pigments, chlorophyll (green), carotenoids (yellow and orange) and phycocyanin (blue).

It is a rich source of provitamin A or beta carotene and superoxide dismutase (SOD) enzyme. Presence of these two antioxidants makes a very effective system for prevention of various harmful effects of heavy metals and chemicals (12). Spirulina is considered as a valuable additional food source of macro and micro nutrients including amino acids, chlorophyll, gamma-linoleic acid, carotenoids, Vitamins A, B_1 , B_2, C, E and trace elements such as iron, iodine, selenium and zinc(13). Spirulina fusiformis possess potent antiviral (14), antioxidant (15,16), anticancer (17) antihyperlipidemic (18), probiotic (19) antidiabetic, antiobesity effect (20) and strengthens immune system (21, 22). These properties were largely related to the Spirulina`s phycobili protein Phycocyanin (23, 24). Spirulina is gaining more attention from medical scientists as a neutraceutical and pharmaceutical (25). Thus, spirulina might offer both as a nutritional as well as therapeutic strategy and it may inhibit lipid peroxidation as it is a cock tail of antioxidants.

The present study has been undertaken to investigate the hepatotoxicity of sodium valproate and to evaluate the modulatory potential of spirulina fusiformis on sodium valproate induced hepatotoxicity and oxidative stress.

Materials and Methods

Animals

Adult male Wistar strain albino rats (200 -250gm) were maintained on standard pellet diet and tap water ad libitum and were kept in polypropylene cages with wood chip bedding under a 12hr light/dark cycle and room temperature 22-24°C. Rats were acclimatized to the environment for 1 week prior to experimental use and were maintained in accordance with the guidelines of the National Institute of Nutrition, Hyderabad, India. The study protocol was approved by the Institutional Animal Ethical Committee.

Chemicals

Spirulina fusiformis was generously supplied as gift sample in the form of spray dried powder by Sunova Pharmaceuticals Ltd., Chennai, Tamil Nadu, India. Sodium valproate was obtained from Torrent., Ahmedabad (India). Aspartic acid, thiobarbituric acid (TBA), butylated hydroxytoluene (BHT) alanine, alpha-ketoglutarate, 2,4-

dinitro phenyl hydrazine (DNPH) were obtained from Sigma Chemical Co (St. Louis, MO-US).

The animals were divided into five groups of seven each and dose of Spirulina was selected from earlier antioxidant studies (26).

Control group (Group-I) Control group received 1% tween 80, orally by gavage daily for 60 days.

Sodium valproate group (Group-II) Sodium valproate at a dose of 18 mg/kg was dissolved in water and administered orally daily between 9 and 10am for 60 days.

Spirulina group (**Group-III)** Spirulina at a dose of 1000mg/kg was suspended in a 1% solution of tween 80 and administered orally daily between 9 and 10am for 60 days.

Prophylactic group (Group-IV) Prophylactic group received sodium valproate at a dose of 18 mg/kg dissolved in water and spirulina at a dose of 1000 mg/kg was suspended in 1% tween 80 and administered orally by gavage daily between 9 and 10 am for 60 days.

Curative group (Group-V) Treated for the first 30 days with sodium valproate at a dose of 18 mg/kg, and received spirulina from 31st day to 60th day at a dose of 1000 mg/kg.

Biochemical Measurements

SGPT (Serum glutamate pyruvate transminase/alanine transaminase) and SGOT (Serum glutamate oxaloacetate transminase/aspartate transaminase) and bilirubin levels were estimated after 60 days of treatment.

1ml of blood was withdrawn by puncturing retro-orbital plexus in a clean and dry test tube under light ether anaesthesia. Blood samples were kept aside for 10 minutes in dry test tubes with out any disturbance. Serum was then separated by centrifuging at 2500rpm for 10 minutes, the levels of SGOT, SGPT, bilirubin, lipid peroxidation and TAS were analyzed and lipid peroxidation was assesed in plasma.

Serum bilirubin level was estimated based on Van den Berg reaction (27), Diazo reagent (0.5ml) reacts with bilirubin in diluted serum (0.2 ml serum + 1.8 ml distilled water) and forms purple coloured azobilirubin, which was measured at 540nm. Activities of serum Aspartate transaminase (AST) and Alanine transaminase (ALT) were assayed by the method of Reitman and Frankel (28). DNPH solution was added to serum with substrate and absorbance was read at 540nm. Activities are expressed as IU/L.

Lipid peroxidation in plasma was estimated colorimetrically by measuring thiobarbituric acid reactive substances by the method of Nichans et al. (29). Plasma was treated with TBA-TCA-HCl reagent and absorbance was read at 535nm. Total antioxidant status in serum was estimate by Blois method (30) by using DPPH solution.

Statistical analysis

All values are expressed as Mean ± S.E. The statistical analysis was carried out using one way analysis of variance (ANOVA) followed by Dunnet's T-test. In all tests, the criterion for statistical significance was $P<0.05$.

Results

Effect of sodium valproate, spirulina and sodium valproate + spirulina on biochemical parameters associated with liver necrosis.

Chronic sodium valproate 18mg/kg treatment significantly increased AST levels ($P<0.001$) when compared to control group. Prophylactic group showed significant increase in AST levels compared to control group ($P<0.001$) and significant decrease in AST levels when compared to sodium valproate group ($P<0.05$). Curative group showed significant increase in AST levels compared to control group ($P<0.01$) and significant decrease in AST levels when compared to sodium valproate group ($P<0.05$) (Table- I).

Chronic sodium valproate 18mg/kg treatment significantly increased the ALT levels ($P<0.001$) when compared to control group. Spirulina alone group showed similar values as that of the control group. Prophylactic group showed increase in ALT levels when compared to control group ($P<0.001$) and significant decrease in ALT levels when compared to sodium valproate group ($P<0.05$).

Curative group showed increase in ALT levels when compared to control group (P<0.001) and significant decrease in ALT levels when compared to sodium valproate group (P<0.05), but the values did not reach normal in either prophylactic or curative group (Table- I).

Chronic sodium valproate 18mg/kg treatement significantly increased the bilirubin levels (P<0.001) when compared to control group. Spirulina alone group showed similar valules as that of the control group. Prophylactic group showed significant increase in bilirubin levels when compared to control group (P<0.001) and significant decrease in bilirubin levels when compared to sodium valproate group (P<0.05). Curative group showed increase in bilirubin levels when compared to control group (P<0.001) and significant decrease in bilirubin levels when compared to sodium valproate group (P<0.05) (Table- I).

Effects of sodium valproate, Spirulina, and sodium valproate + Spirulina on lipid per oxidation.

Chronic sodium valproate 18mg/kg treatment significantly increased the lipid peroxidation (P<0.001) when compared to control group. Prophylactic group showed significant incrase in lipid peroxidation levels when compared to control group (P<0.001) and significant decrease in the lipid peroxidation levels when compared with sodium valproate group (P<0.05). Curative group showed significant increase in lipid peroxidation levels when compared to control group (P<0.001) and significant decrease in the lipid peroxidation levels when compared with sodium valproate group (P<0.05) (Table- II).

Effects of sodium valproate, Spirulina, and sodium valproate + Spirulina on total antioxidant status.

Chronic sodium valproate 18mg/kg treatment significantly decreased the total antioxidant levels (P<0.001) when compared to control group. Prophylactic group showed decrease in total antioxidant levels when compared to control group (P<0.001) and showed significant increase of the same when compared with sodium valproate group (P<0.05). Curative group showed significant decrease in total antioxidant levels when compared to control group (P<0.001) and showed significant increase in the same when compared with sodium valprote group (P<0.05) (Table- II).

Table 1: Effect of chronic treatment of sodium valproate and spirulina on serum **AST, ALT** and **Bilirubin** levels in experimental rats.

GROUP	AST (IU/L)	ALT (IU/L)	Bilirubin (mg/dL)
GROUP-I (Control)	140.5 ± 0.567	39.43 ± 1.00	0.451 ± 0.029
GROUP-II (S.V -18 mg/kg)	253.9 ± 4.836[a]	69.07 ± 0.557[a]	0.989 ± 0.018[a]
GROUP-III (Spirulina-1000mg/kg)	138.9 ± 0.359	37.36 ± 0.582	0.401 ± 0.012
GROUP-IV [S.V + Spirulina (P)]	170.4 ± 1.462 [a,b]	44.99 ± 1.343 [a,b]	0.601 ± 0.10 [a,b]
GROUP-V [S.V + Spirulina (T)]	201.0 ± 2.330 [a,b]	53.87 ± 1.064[a,b]	0.789 ± 0.06 [a,b]

Values are expressed as mean ± SEM of 7 animals; [a]P<0.001vs control group, [b]P<0.05 vs S.V group. S.V- Sodium valproate, P-Prophylactic group, T- Curative group.

Table II : Effect of chronic treatment of sodium valproate and spirulina on plasma **lipid peroxidation** and serum total antioxidant levels in experimental rats.

GROUP	Lipid peroxidation (mmol/dL/h)	TAS (mmol/L)
GROUP-I (Control)	9.507 ± 0.239	0.836 ± 0.114
GROUP-II (S.V -18 mg/kg)	1.418±0.247 [a]	0.515 ± 0.018[a]
GROUP-III (Spirulina-1000mg/kg)	8.503 ± 0.155	0.948±0.011
GROUP-IV [S.V + Spirulina (P)]	1.06 ±0.042 [a,b]	0.765 ± 0.01 [a,b]
GROUP-V [S.V + Spirulina (C)]	1.290± 0.791[a.b]	0.624 ± 0.01 [a,b]

Values are expressed as mean ± SEM of 7 animals; [a]P<0.001vs control group, [b]P<0.05vs S.V group. S.V- Sodium valproate, P-Prophylactic group, C-Curative group, TAS- Total antioxidant status

Discussion

The present study evaluates the protective effect of spirulina against liver damage and oxidative stress induced by sodium valproate in male Wistar rats. Sodium valproate at a dose of 18mg/p.o/Kg showed severe hepatic damage and oxidative stress associated with marked increase in the serum activity of aminotrasferases, bilirubin, lipid peroxidaion. Free radicals and lipid peroxidation cause acute lethal damage of hepatocytes in ischemic hepatitis (6). VPA-treated rats showed a significant increase in the levels of AST, ALT, bilirubin, lipid peroxidation and TAS. Serum AST, ALT, and bilirubin are the most sensitive markers employed in the diagnosis of hepatic damage as these are cytoplasmic in location and are released into the circulation after cellular damage (31).

Hepatotoxicity due to VPA appears to be from the production of toxic metabolites, the most toxic VPA metabolite, 4-ene-VPA, a product of omega oxidation by the cytochrome p-450 enzyme systems (7). Cytochrome P-450 enzymes mediate the production of an oxidative metabolite of valproic acid capable of generating coenzyme derivatives. Production and accumulation of these derivatives inhibits mitochondrial oxidation via depletion of free coenzyme A and carnitin concentrations (32). Sodium valproate is metabolized by fatty acid β-oxidation and conjugated by glucuronic acid with a minimal contribution by omega oxidation, chronic or high-dose sodium valproate therapy inhibits β-oxidation. This causes a decrease in benign metabolities of β-oxidation and increases toxic metabolites of omega oxidation in compensation, which cause injury to bile duct epithelium (33).

A number of studies have reported oxidative stress in patients (11,34, 35,36,37) and in animal models treated with valproic acid (38, 8). Earlier studies demonstrated that VPA caused dose-dependent toxicity to rat hepatocytes (39, 40). In support of the oxidative stress hypothesis of VPA-hepatotoxicity, lipid peroxidation is involved in VPA-hepatotoxicity, the antioxidants, vitamin C, alpha-tocopherol, N, N-diphenyl-phenylenediamine, melanin and α-ketoglutarate conferred protection against VPA toxicity in rat hepatocyte cultures (41, 7). These findings further confirm the hypothesis that VPA is associated with oxidative stress.

Cells are protected from oxygen-derived radical injury by naturally occurring free- radical scavengers and antioxidant pathways, including vitamins A, C, E, SOD, catalase and glutathione peroxidase when these protective mechanisms are overwhelmed, however, host tissues become susceptible to damage by oxygen radicals that peroxidate lipids and disturb cell membrane function (42). Oxidative stress is an imbalance between the production of oxidants and the respective defence system of an organism. Oxidants, such as reactive oxygen species, and many others, damage biomolecules by chain reactions in which one radical can induce the oxidation of a large number of substrate molecules (43, 44). MDA content is a direct indicator of the extent of lipid peroxidation due to oxidative stress. Lipid peroxidation is the interaction between molecular oxygen and polyunsaturated fatty acids by products of lipid peroxidation cause marked alteration in the structural integrity and function of cell membranes. An imbalance between antioxidant defence mechanisms and lipid peroxidation processes results in cell and tissue damage (45). Cytotoxic activity of sodium valproate is the result of the generation of hydrogen peroxide and the production of highly reactive hydroxyl free radicals (46). Toxic forms of activated oxygen react with cellular components resulting in oxidation of proteins, nucleic acids, as well as lipids leading further to inactivation of enzymes, disruption of membranes, mutation of genes and ultimately cell death (42, 47).

Treatment with spirulina significantly decreased the activities of AST, ALT, bilirubin, and increased TAS in serum by decreasing lipid peroxidation levels in plasma suggesting that they offer protection by preserving the structural integrity of hepatocellular membrane against sodium valproate induced heaptotoxicity and oxidative stress. The protective efficacy of spirulina fusiformis may be due to the presence of several active components. The active component found in spirulina may provoke the activity of free radical scavenging enzyme systems and render protection against sodium valproate induced liver damage and oxidative stress. The protective role of spirulina may be attributed to the presence of beta carotene (48, 49), Vitamin C, E (50), super oxide dismutase enzyme and selenium (51). Spirulina is the richest beta carotene food, non-enzymatic antioxidants such as carotenoids, play an important role in the cellular response to oxidative stress by reducing ROS at different sites to enhance antioxidant protection (52). Beta carotene quenches singlet oxygen and a scavenges free radicals, reduce cell damage, especially

the damage to DNA molecules, thus playing the role in the repair and regeneration process of damaged hepatocyte cells (53, 54).

Vitamin C present in spiruluna acts as an antioxidant by quenching hydroxyl and superoxide radical reactivity (55). Vitamin E present in spirulina traps lipid peroxyl and other radicals and effectively inhibits the peroxidation of cellular membranes and maintains GSH and ascorbic acid levels in damaged tissue by inhibiting free radicals formation (56, 57, 58, 59). Both in vivo and in vitro studies have showen vitamin A to be a potent membrane lipid antioxidant. Vitamin A stabilises membrane and reduces lipid peroxidation (60, 61). Phycocyanin pigment present in spirulina significantly inhibits hydroxyl, alkoxyl, peroxyl radicals, induced lipid peroxidation in rat liver microsomes (62,63), scavenges superoxides, peroxy dinitrate thereby reduces peroxy dinitrate induced oxidative damage to DNA (62,63,64), is 20 times more potent than ascorbic acid (65). Phycocyanobilin (a component of phycocyanin) more potent antioxidant than α-tocopherol on molar basis (66). Superoxide dismutase of spirulina is a mitochondrial enzyme, dismutases superoxide radicals there by prevents the formation of hydroxyl radicals and prevents tissue damage (50, 67, 68). Since sodium valproate induced hepatotoxicity involves free radical production, the antioxidant and free radical scavenging property of sprirulina would have provided the protection against hepatic damage.

In the present study, administration of spirulina significantly altered the level of AST, ALT, bilirubin, lipid peroxidation and TAS in prophylactic and curative groups. Pretreatment with spirulina along with valpraote attenuated the levels of hepatotoxic and oxidative stress markers in prophylactiv group. In recent years there is increasing interest in assessing the total antioxidant capacity because of the difficulty in measuring each antioxidant component separately as well as the interactions among different components. Spirulina has building blocks such as minerals, phytonutrients, antioxidants and polysaccharides that trigger enzyme systems and increases total antioxidant status and induces the activity of immune system and builds up both the cellular and humoral arms of the immune systems and thus improving their ability to function inspite of stresses from environmental toxins and infectious agents (14, 22).

In the present study spirulina when given in combination with sodium valproate, significantly increased the levels of TAS and reduced sodium valproate toxicity which in turn is reflected by significant decrease in activity of serum transaminases, bilirubin, and lipid peroxidation.

The present findings are corroborate with the previous reports, Vadiraja et al., (69) reported that an extract of spirulina showed protection against carbon tetra chloride induced hepatotoxicity in rats. The phycocyanin and other antioxidants present in the extract account for the hepatoprotective effect. Phycocyanin showed protection against heaptotoxins in rats, due to its antioxidant activity. Our findings are in agreement with previous investigations which reported hepatoprotective activity by various antioxidants like, melatonin (9), tetrahydrocurcumin (70), Vitamin E, EGb 761 (71) and alpha-ketoglutarate (34, 35). Our results are further supported by several studies which reported spirulina inhibited lipid peroxidation (72, 73), showed protection against cadmium (74) and CCl_4 induced hepatotoxicity (75), cisplatin and urethane induced genotoxicity (76) and haloperidol induced tardive dyskinesia and oxidative stress in rats (77).

In the present study, it was observed that the sodium valproate increased significantly the levels of AST, ALT and bilirubin, markers of hepatotoxicity and lipid peroxidation, marker of oxidative stress in the rats. Treatment with spirulina decreased the markers of hepatotoxicity and increased the antioxidant status. It indicates that sodium valproate induces oxidative stress and the spirulina has the antioxidant capacity which decreases the hepatotoxic markers. Spirulina exerts significant protection against sodium valproate induced toxicity by its ability to ameliorate the lipid peroxidation through the free radical scavenging activity, which enhanced the levels of antioxidant defence system. It indicates need of antioxidant in the treatment of epilepsy with sodium valproate.

References

1. Werth B, Kuhn M, Hartmann K, Kobler E, Reinhart WH. Drug-induced liver disease: experiences of the Swiss Center for Adverse Drug Effects. J Suisse Med.1993, 123 : 1203 - 6.

2. Dreifuss FE, Santilli N, Langer DH, Sweeny KP, Moline BA, Meander KB. Valproic acid hepatic fatalities: a retrospective review. Neurology. 1987, 37 : 397- 400.
3. Zimmerman HJ, Ishak KG. Sodium valproate induced hepatic injury. Analysis of 23 fatal cases. Hepatology. 1982, 2 : 591-597.
4. Eadie MJ, Hiper WD, Dickinson RG. Sodium valproate-associated hepatotoxicity and its biochemical mechanisms. Med Toxicol. 1988, 3 : 85 -106.
5. Zaret BS, Beckner RR, Marini AM et al. Sodium valproate induced hyperammonemia without clinical hepatic dysfunction. Neurology. 1982, 32 : 206-208.
6. Poli G. Liver damage due to free radicals. Br Med Bull.1993, 49 : 604-620.
7. Jurima-Romet M, Abbot F, Tang W, Huang HS, Whitehouse LW. Cytotoxicity of unsaturated metabolities of valproic acid and protection by vitamins C and E in glutathione-depleted rat hepatocytes. Toxicology. 1996, 112 : 69-85.
8. Raza M, Al-bakairi AM, Agel AM, Qurashi S. Biochemical basis of sodium valproate hepatotoxicity and renal tubular disorder. Time dependence of peroxidative injury. Pharmacol.Res. 1997, 35 :153-157.
9. Siddique MAA, Nazmi AS, Razia K, Karim S, Pal SN, Pillai KK. Oxidative damage in mice liver induced by sodium valproate: protection by melatonin. Ind. J. Pharmacol. 1999, 31: 427- 430.
10. Klee S, Johanssen S, Ungemach FR. Evidence for a trigger function of valproic acid in xenobiotic-induced hepatotoxicity. Pharmacol.Toxicol. 2000, 87: 89-95.
11. Graf WD, Oleinik OE, Glauser TA, Maertens P, Eder DN, and Pippenger CE. Altered antioxidant enzyme activities in children with a serious adverse experience related to valproic acid therapy. Neuropediatrics. 1998, 29 :195-201.
12. Chamorro GM, Salazar L, Favila and H.Bourges. Effect of Spirulina maxima consumption on reproduction and pre and postnatal development in rats. Food Chem Toxicol.996, 34 : 353.
13. Mazo VKIV. Gmoshinskii and IS Zilova. Microalgae. Spirulina in human nutrition. Vopr. Pitan. 2004, 73 :45-53.
14. Hayashi K, Hayashi T and Kojima I. A natural sulfated polysaccharide, calcium spirulin, isolated from Spirulina platensis : invitro and ex vivo evaluation of anti-herpes simplex virus and antihuman immuno-deficiency virus activities. AIDS Res. Hum. Retroviruses. 1996, 12 : 1463-1471.

15. Mirinda MS, Cintra RG, Barros SM, Mancini-Fitho J. Antioxidant activity of the microalga spirulina maxima. Braz Med Biol Res,1998, 31 :1075-1079.

16. Rimbau V, Camis A, Romay C, Gonazalez R, Pallas M. Protective effect of C-phycocyanin against kainicacid-induced neuronal damage in rat hippocampus. Neruoscience Letters, 1999, 27 : 75-78.
17. Qureshi MA, Garlich JD. and Kidd MT. Dietary Spirulina platensis enhances humoral and cell mediated immune functions in chickens. Immunopharmacol. Immunotoxicol.1996, 18 : 465 - 476.
18. Iwata K, Inayama, Kato T. Effects of Spirulina platensis on fructose-induced hyperlipidemia in rats. J Japn Soc Nutr Food Sci. 1987, 40 : 463-467.
19. Parada JL, De caire G. De Mule Mc deno Cano MM. Lactic acid bacteria growth promoters from spirulina platensis. Int. J. Food Microbiol.1988, 45 : 225-228.
20. Becker EW, Jakover B, Luft D. Schmuelling RM. Clinical and Biochemical evaluations of the alga spirulina with regard to its application in the treatment of obesity: double- blind cross-over study. Nutr. Rep. Int.1986, 33 : 565-574.
21. Qureshi MA, Kidd M, Tand Ali RA. Spirulina platensis extract enhances chicken macrophage fuctions after in vitro exposure. J. Nutr. Immunol.1995, 3: 35- 45.
22. Liu L, Guo B, Ruan J, Dai X, Chen L, Wu B. Study on effect and mechanism of polysaccharides of Spirulina platensis on body immune functions improvement. Marine SCi.1991, 6 : 44-49.
23. Estrada JE, P Bermejo Bescos and AM Villar del, Fresno. Antioxidant activity of different fractions of Spirulina platensis protean extract. Phyto chemistry. 2001 61 : 12-16.
24. Wu LC, JA Ho, MC. Shieh and IW Lu. Antioxidant and antiproliferative activities of Spirulina and Chlorella water extracts. J.Agric. Food Chem. 2005, 53 : 4207-4212.
25. Khan Z, P Bhadouria and PS Bisen. Nutritional and therapeutic potential of Spirulina. Curr. Pharm. Biotecnol. 2005, 6 : 373-379.
26. Iyyapu Krisna Mohan, Mahmood Khan, Jagdish Chandra Shobha, Madireddy Umamaheswara Rao Naidu, Aruna Prayag, Perjannan Kuppusamy and Vijay Kumar Kutala. Protection against Cisplatin induced nephrotoxicity by Spirulina in rats. Cancer Chemotherapy and Pharmacology. 2006, 6 : 58.

27. Malloy E, Evelyn K. The determination of bilirubin with the photoelectric colorimeter. J. Biol. Chem.1937, 119, 481 - 485.
28. Reitman S, Frankel ASA. Colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. Amer. J. Clin.Pathol.1995, 28 : 56.
29. Nichans WG, Samuelson D. Formation of malondialdehyde from phospholipids arachidonate during microsomal lipid peroxidation. Eur J Biochem.1959, 6 : 126-130.
30. Blios MS. Antioxidant determination by the use of stable free radical. Nature.1958 26 :11-14.
31. Sallie R, Tredger JM, Willam R. Drugs and the Liver. Biopharmaceutical Drug Dispos.1991, 12 : 251-259.
32. Chung set al. Alterations in the carnation metabolism in epileptic children treated with valproic acid. J Korean. Med. Sci. 1997, 12 : 553-8.
33. Fromenty B, Pessayre D. Inhibition of mitochondrial beta-oxidation as a mechanism of hepatotoxicity. Pharmacol Ther: 1995, 67:101-154.
34. Cengiz M, Yuksel A, and Seven M. The effects of carbamazepine and valproic acid on the erythrocyte glutathione, glutathione peroxidase, super oxide dismutase and serum lipid peroxidation in epileptic children. Pharmacol. Res. 2000, 41: 423-425.
35. Hurd RW, Van Rinsyelt HA, Wilder BJ, Karas B, Maenhaut, W, and De Reu L. Selenium, Zinc and copper changes with valproic acid. Possible relation to drug side effects. Neurology 1984, 34 :1393-1395.
36. Yuksel A, Cengiz M, Seven M, Ulutin T. Changes in the antioxidant system and hepatic enzymes in epileptic children receiving antiepileptic drugs. J. Child. Neurol. 2001,16 : 603-606.
37. Martinez-Ballesteros C, Pita-Calandre E, Sanches-Gonzalez Y, et al. Lipid peroxidation in adult epileptic patients treated with valproic acid. Rev Neurol. 2004, 38 :101-106
38. Cotariu D, Evans S, Zaidman JL and Marcus O. Early changes in hepatic redox homeostasis following treatment with a single dose of valproic acid. Biochem. Pharmacol. 1990, 40 : 589-593.
39. Kingsley E, Gray P, Tolman KG, and Tweedale R. The toxicity of metabolites of sodium valproate in cultured hepatocytes. J. Clin. Pharmacol. 1983, 23 : 178-185.
40. Vincent Tong, Xiao Wei Teng, Thomas KH, Chang and Frank S, Abbott. Valproic Acid II : Effects on Oxidative Stress, Mitochondrial Membrane Potential, and Cytotoxicity in Glutathione-

Depleted Rat Hepatocytes. Toxicological Sciences. 2005, 86 : 436 – 443.
41. Buchi KN, Gray PD, Rollins DE, and Tolma KG. Protection against sodium valproate injury in isolated heaptocytes by alpha-tocopherol and N, N-diphenyl-p-phenylenediamine. J. Clin. Pharmacol. 1984, 24 : 148-154.
42. Imoly JA, Linn S. DNA damage and oxygen radical toxicity. Sciene 1988, 240 : 1302-1309.
43. Choi BH. Oxygen, antioxidants and brain dysfunction. Yonsei Med. J. 1993, 34,1-10.
44. Delanty N, Dichter MA. Oxidative injury in the nervous system. Acata Neurol. Scand. 1998, 98 :145-153.
45. Gutteridge JMC and Halliwell B. The measurement and mechanism of lipid peroxidation in biological systems. Trends Biochem Sci. 1992 , 15 : 129-135.
46. Tabatabaci AR, Abott FS. Assessing the mechanism of metabolism-dependent valproic acid induced in vitro cytotoxicity. Chem. Res. Toxicol.1993, 12, 323-330.
47. Halliwell B, Gutteridge JMC. Free Radicals in Biology and Medicine. Oxford Sciene Publications. Oxford. 2nd CD, 1989.
48. Prescot GW. How to know the fresh water algae? 3rd ed., WC. Brownn Company Publishers, Iowa, U.S.A. 1978. p.182..
49. Seshadri CV, Umesh BV and Manoharan R. Beta carotene studies in Spirulina. Bio Res Tech. 1991. 38 :111-113.1991.
50. Mathew B, Sankarnarayanan R, Nair PP, Varghese P, Somanthan T, Amma BP, Amm NS and Nair MK. Evaluation to chemoprevention of oral cancer with spirulina fusiformis. Nutr. Cancer.1995, 24, 194 -202.
51. Henriksen R. Earth food Siprulina. Cited from Recolina Ltd. Ronore enterprises Inc. Launa Beach, California pp. 1989, 27-65.
52. Foote CF, Chang YC and Denny RW. Chemistry of singlet oxygen. X. Carotenoids quenching parallels biological protection. J. Am. Chem. Soc. 1970, 92 : 5216 - 5219.
53. Krinsky NI and Deneke SM. Interaction of oxygen and oxyradicals with carotenoids. J. Nat. Cancer Inst. 1982, 69: 205 - 210.
54. Luxia AS, Monica S, Ornella C, Plizzala B, Laura R, Livia B, Anio M. and Ennio P. Effect of betacarotene on cellcycleprogression of human fibroblasts. Mutagenesis, 1996, 17 : 2395 - 2401.
55. Adrianne Bendich. Antioxidant Micronutrients and Immune Responses. Micronutrients and Immune Fucntions.A Bendich and R.K. Chandra (eds) New York Academy of Sciences, New York, 1990, pp-175.

56. Duval C, Poelman M Scavenger effect of vitamin E and derivatives on free radicals generated by photo irradiated phenomelanin. J Pharm Sci 1994, 84 :107-110.
57. Kulkarni AP, Byczkowski JZ. Introduction to biochemical toxicology, 2nd ed. Appleton and Lange, Connecticut.1994, pp 103-105.
58. Bickford PC, Gould T, Briederick L, Chadman K, Pollock A, Young D, Shukitt-Hale B, Josesph J. Antioxidant-rich diets improve cerebellar physiology and motor learning in aged rats. Brain Res. 2000, 866 : 211-217.
59. Gemma C, Mesches MH, Sepesi B, Choo K, Holmes DB, Bickford PC. Diet enriched in foods with high antioxidant activity reverse age-induced decreases in cerebellar-adrenergic function and increases in proinflammatory cytokines. J Neurosci. 2002 , 22 : 6114 - 6120.
60. Tsuchiya M, Scita G, Thompson DFT, Kagan VE, Livrea MA, Packer L. Retinoids and carotenoids are peroxyl radical scavengers. Retinoids. Progress in Research and clinical Applictions. Livrea MA, Packer Leds. New York : Marcel Dekker. 1993, 6: 525-36.
61. Ciaccio M, Valenza M, Tesourure L, Bongiorno A, Albiero R. Vitamin A inhibits doxorubicin induced membrane lipid peroxidation in rat tissues in vivo. Arch biochem.1997, 6 : 554-7.
62. Bhat VB, Madyastha KM. C-phycocyanin : a potent peroxyl radical scavenger in vivo and in vitro. Biochem Biophys Res Commun. 2000, 285 : 262-266.
63. Bhat VB, Madyastha KM. Scavenging of peroxynitrite by phycocyanin and phycocyanobilin from Spirulina plantensis : protection against oxidative damage to DNA. Biochem Biophys Res Commun. 2001, 285 : 262 - 266.
64. Romay C, Armesto J, Remirez D, Gonzalez R, Ledon N, Garcia I. Antioxidant and anti-inflammatory properties of C-phycocyanin from blue-green algae. Inflamm Res.1998, 47 : 36-41.
65. Romay C, Gonzalez R. Phycocyanin is an antioxidant protector of human erythrocytes against lysis by peroxy radicals. J Pharm Pharmacol. 2000, 52 : 367-368.
66. Hirata T, Tanaki M, Ooike M, Tsunomura T, Sagakuchi M. Antioxidant activities of phycocyanobilin prepared from Spirulina platensis. J Appl Phycol. 2000, 12 : 435-439.
67. Girardi G and Elias MM. Mercuric chloride effects on rat renal redox enzymes activities: SOD protection. Fredd Radic. Biol. Med. 18, 61-66.

68. Yao JK, Reddy R, Elhinny LG, Vankammen DP. Effects of haloperidol on antioxidant defense system enzymes in schizophrenia through its antioxidant properties. J. Psychiatric Res. 1998, 32 : 385 -391.
69. Vadiraja B, Gaikwad N, Madyastha K. Hepatoprotective effect of C-phycocyanin : protection for carbon tetrachloride and R - (+)-pulegone-mediated hepatotoxicity in rats: Biochem Biophys Res Commun. 1988, 4 : 428 - 431.
70. Leelavinothan Pari D, Roalin Amali. Protective role of tetrahydrocurcumin (THC) an active principle of turmeric on chloroquine induced hepatotoxicity in rats. J. Pharm Pharmaceut Sci. 2005, 8 :115-123.
71. Coskun O, Yakan B, Oztas S, Sezen A. Antioxident and Hepatoprotective Activity of Vitamin E and EGb 761 in Experimental Endotoxemic Rats. J. Med. Sci. 2000. 5 : 227- 432.
72. Gonzalez R, Rodriguez S, Romay C, Gonzalez A, Armesto J, Remirez D, Merino N. Anti-inflammatory activity of phycocyanin extract in acetic acid-induced colitis in rats. Pharmacol Res. 1999, 39 :1055 -1059.
73. Remirez D, Gonzalez R, Merino N, Rodriguez S, Ancheta O. Inhibitory effects of Spirulina in zymosan- induced arthritis in mice. Mediators Inflamm.1987, 11 : 75 -79.
74. Amar Amini, Alaaeldin A. Hamzai, Sayel Daoud and Waleed Hamzai. Spirulina protects against cadmium-induced hepatotoxicity in rats. Am J of Pharma.Toxicol, 2006, 2 : 21- 25.
75. Torres-Duran PV, Miranda-Zamora R, Paredes -Carbajal MC, Mascher JC. Spirulina maxima prevents induction of fatty liver by carbonntetrachloride in the rat. IUBMB Life, 1988 , 44 : 787 - 793.
76. Premkumar K, SK Abraham, ST Santhiya and A Ramesh. Protective effect of Spirulina fusiformis on chemical-induced genotoxicity. Fitoterapia, 2004, 75 : 24 -31.
77. Thaakur SR, Jyothi B. Effect of Spirulina maxima on the haloperidol induced tardive dyskinesia and oxidative stress in rats. 2007, J. Neural.Transm. 114 : 1217-1225.