RESEARCH PUBLICATIONS ARISING FROM THE PRESENT STUDY

1. Upasani, C. D. and Balaraman, R. (2001). Protective Effect of *Spirulina* on Lead Induced Deleterious Changes in the Lipid Peroxidation and Endogenous Antioxidants in Some Organs of Rats.
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2. Upasani, C. D. and Balaraman, R. (2001). Effect of vitamin E, Vitamin C and *Spirulina* on the levels of membrane bound enzymes and lipids in some organs of rats exposed to lead.
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3. Upasani, C. D., Khera, A. and Balaraman, R. (2001). Effect of lead with vitamin E, vitamin C or *Spirulina* on malondialdehyde, conjugated dienes and hydroperoxides in rats.
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Protective Effect of *Spirulina* on Lead Induced Deleterious Changes in the Lipid Peroxidation and Endogenous Antioxidants in Some Organs of Rats.

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Running Title

Spirulina, a blue green algae (Oscillotoreaceae), is a rich source of some antioxidant vitamins.

Lead acetate (100ppm) showed a significant increase in the lipid peroxidation or malondialdehyde formation and decreased the levels of endogenous antioxidants. *Spirulina* treatment to the lead exposed rats significantly decreased the level malondialdehyde formation and restored the levels of endogenous antioxidants like SOD, CAT and GSH in liver, lung, heart and kidney. Further, no significant changes were observed in brain.

Except brain, *Spirulina* has been found to have a protective antioxidant effect on the organs of animals exposed to lead.

ABSTRACT

The present study is aimed to investigate the protective effect of *Spirulina* on the lead-induced changes in the levels of lipid peroxidation and endogenous antioxidants in liver, lung, heart, kidney and brain of rats. One group of rats received lead (100ppm) dissolved in double deionized water and the other group received lead along with *Spirulina* (1500mg/kg) for thirty days. The levels of lipid peroxidation or malondialdehyde formation and endogenous antioxidants such as superoxide dismutase, catalase, reduced glutathione were measured. The elemental lead levels were also measured in liver, lung, heart, kidney and brain of rats in all experimental groups. In liver, lung, heart and kidney of lead exposed animals, there was a significant ($p<0.001$) increase in the lipid peroxidation and decrease in the levels of endogenous antioxidants. Although, *Spirulina* did not affect the deposition of lead in organs except brain, simultaneous administration of *Spirulina* to lead exposed animals significantly ($p<0.001$) inhibited the lipid peroxidation and restored the levels of endogenous antioxidants to normal in liver, lung, heart and kidney.

To conclude, *Spirulina* had a significant effect on scavenging the free radicals thereby protecting the organs from damage caused by the exposure of lead. Further, *Spirulina* shown the significant ($p<0.05$) decrease in the deposition of lead in brain.

Key words: *Spirulina*, lead acetate, MDA, SOD, catalase, GSH.

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INTRODUCTION

The toxic effects of lead have long been recognized (Liu-Fu, 1982) and to date remains a major public health problem (Graziano et al., 1985). It has been reported that, primary sources responsible for lead exposure include food, water and inhalation (Bryce-Smith and Stephens, 1981; Friberg, et al., 1979). Once the ecosystem is contaminated with lead or any other heavy metal they remain a potential threat for a long time. Exposure to environmental lead is known to affect various organ systems (Goyer, 1993; Friberg, et al., 1979; Goyer and Rhine, 1978). Absorption of inorganic lead is reported to cause biochemical and metabolic toxicity (Stankovic, 1971; Murthy and Rhea, 1971). Lead is known to inhibit large number of processes, which depend on sulfhydryl groups e.g. various enzymes, Ca^{2+} channels etc. thereby showing diverse toxicity. One of the causes of lead toxicity might be its impact on the free radical release like hydroxyl radical (Ding, et al., 2000) and inhibition of certain enzymes like nitric oxide synthase activity (Garcia-Arenas et al., 1999) in vital organs.

The endogenous antioxidant enzymes like Superoxide dismutase (SOD) (superoxide: superoxide oxido reductase, EC-1.15.1.1) converts the superoxide free radical anion to hydrogen peroxide. Catalase (CAT) (hydrogen peroxide: hydrogen peroxide oxido reducatase, EC-1.11.1.6) is capable of scavenging hydrogen peroxide radical, which is formed during various biochemical and metabolic reactions. The tripeptide Glutathione (GSH) (γ - glutanyl - cysteinyl - glycine) is involved in many important cellular functions, ranging from the control of physicochemical properties of cellular proteins and peptides to the detoxification of free radical (Meister and Anderson, 1983). Recent studies have reported that, lead might be inducing various abnormalities like hypertension (Ding, et al., 2000), lipid peroxidation (LPO) (Patra and Swarup, 2000), inhibition of heme biosynthesis (El-Missiry, 2000) which are closely related to enhanced activity of reactive oxygen species or free radicals.

Spirulina fusiformis, a blue-green algae, (Oscillotoreaceae) is rich in all the three types of micronutrients proteins, lipids and carbohydrates. Besides these, some more elements like zinc, magnesium, manganese, selenium and some vitamins like β-carotene, riboflavin, cyanocobalamine, α-tocopherol, α-lipoic acid are also present (Sheshadri and Umesh, 1992). *Spirulina* is also believed to be an external source of a vital antioxidant enzyme superoxide dismutase (SOD) (Henrikson, 1989).

The primary objective of the present study is to explore the effect of lead on the levels of lipid peroxidation and endogenous antioxidants like SOD, CAT and GSH. Secondly, it is also aimed to find out the scope of exogenous antioxidant, *Spirulina* on experimentally altered lipid peroxidation and endogenous antioxidant levels by lead in the organs like liver, lung, heart, kidney and brain of rats. Thirdly, the levels of lead in these organs along with behavioral studies in all experimental groups were also explored.

MATERIAL AND METHODS

(a) Animals:

Adult albino rats (Wistar strain) of either sex weighing 150-225gm were maintained at 25±3°C in a well-ventilated animal house under

natural photoperiod conditions in large polypropylene cages.

(b) Sources of Chemicals, Metal and *Spirulina*:

All the reagents of analytical grade were used in the present study. SOD, CAT reference standards and reduced glutathione were obtained commercially from Sigma Chemicals, St. Louis, U.S.A. Lead (in the form of lead acetate) was obtained from SD fine Chemicals, Bhoisar, Mumbai, India. *Spirulina fusiformis* (in powder form) was obtained from Indon Healthcare Ltd., Aslali, Ahmedabad, India.

(c) Experimental Procedure:

Lead acetate was dissolved in double deionized water (100ppm) and given *ad libitum* (Schroeder and Vinton, 1962). *Spirulina* was mixed (1500mg/kg) in food. The mixing of Sprirulina in food was done after observing the daily food intake of rats for the period of fifteen days. The dose of *Spirulina* was calculated by measuring the amount of food given and the amount of food left out in the container. The food consisted of untreated seed rye flour (60%), powdered skimmed milk (30%), corn oil (9%), sodium chloride (1%) with added iron and vitamins (Schroeder et al., 1963). All the animals were treated for thirty days.

Animals were divided into four groups of six animals in each groups. **Group-I (Control group):** Received normal food and double deionized water (DDW). **Group-II (LA):** Received normal food and 100ppm lead acetate dissolved in DDW. **Group-III (SP):** Received *Spirulina* (1500mg/kg) mixed food and DDW. **Group-IV (SP+LA):** Received *Spirulina* (1500mg/kg) mixed food along with 100ppm-lead acetate dissolved in DDW.

At the end of treatment of thirty days, rats were deprived of food overnight and all the animals were sacrificed by carotid bleeding after being anaesthetized with urethane (120mg/100gm of body weight, ip). The heads of the rats were frozen immediately at 0°C by keeping it in deep freezer. Following the procedure of Uma and Ramakrishnan (1983) brain was isolated. Liver, lung, heart, kidney and brain were collected by using precooled petridishes and were blotted free of blood and tissue fluid. After being weighed the organs were cross-chopped with surgical scalpel into fine slices, chilled in the ice-cold 0.25M sucrose solution and then blotted on a filter paper. The tissue were then minced and homogenized in chilled 10mM Tris-HCl buffer (pH 7.4) at a concentration of 10% w/v with 25 strokes of tight Teflon homogenizer at a speed of 2500 rpm. Prolonged homogenization under hypotonic conditions disrupted the ultrastructure of cells so as to release soluble proteins and leave membrane and non-vascular matter in a sediment form. The homogenates were centrifuged at 100000 X g at 4°C for 20 minutes (Ramana and Kohli, 1999; Bopanna et al., 1998). The clear supernatant was immediately used for the enzyme assay. The estimation of lipid peroxidation or malondialdehyde (MDA) formation (Slater and Sawyer, 1971), superoxide dismutase (Misra and Fridovich, 1972), catalase (Sinha, 1972) and glutathione (Moran et al., 1979) was done by earlier reported methods. The above-mentioned endogenous antioxidants were colourimetrically estimated by using Hitachi U-2000, ultra-violet double beam spectrophotometer. The estimation of tissue levels of lead was done ,by Atomic absorption spectrophotometer (Perkin-Elmer, 2380) using concentrated nitric acid digestion method (Greenberg et al., 1992).

STATISTICS

The Mean ± SEM values were calculated for each group to determine the significance of inter group difference. Each parameter was analyzed separately by using one way analysis of variance (ANOVA) with Huynh-Feldt Epsilon modification of degrees of freedom to correct the departure from homogeneity. For finding out the differences between the groups Student 't' test was used. A 'p' value of <0.05 was considered to be significant.

RESULTS

In the present investigation no mortality was observed in animals of any groups exposed to lead or *Spirulina* alone or in combination during the treatment period of thirty days.

The MDA levels in liver, lung, heart and kidney were increased significantly ($p<0.001$) in group II (LA) when compared with group I (control). The MDA levels in liver, lung, heart and kidney were significantly ($p<0.001$) decreased in group IV (SP+LA) as compared to group II (LA). (Table 1).

Table 2 indicates the levels of SOD, CAT and GSH in the liver, lung, heart and kidney. The SOD levels in liver, lung, heart and kidney were decreased significantly ($p<0.001$) in group II (LA) when compared with group I (control). *Spirulina* administration to lead exposed animals showed a significant ($p<0.001$) increase in SOD levels in liver, lung, heart and kidney in group IV (SP+LA) as compared to group II (LA).

The CAT levels in liver, lung, heart and kidney were decreased significantly ($p<0.001$) in group II (LA) when compared with groupI (control). The CAT levels in liver, lung, heart and kidney were significantly ($p<0.001$) increased in group IV (SP+LA) as compared to group II (LA). The GSH levels in liver, lung, heart and kidney were decreased significantly ($p<0.001$) in group II (LA) when compared with group I (control). The GSH levels in liver, lung, heart and kidney were significantly ($p<0.001$) increased in group IV (SP+LA) as compared to group II (LA). Except brain, *Spirulina* elevated the levels of SOD, CAT and GSH in liver, lung, heart and kidney of lead exposed animals indicates the protective antioxidant effect.

The nitric acid digested tissue samples were subjected to atomic absorption spectrophotometer. The results indicated that, tissue lead levels were significantly ($p<0.001$) increased in liver, lung, heart, kidney and brain in group II animals as compared to group I. There was a significant ($P<0.001$) reduction in lead levels in brain of group IV animals as compared to group II animals. However, in liver, lung, heart and kidney no significant changes in lead levels were observed in group IV as compared to group II animals (Table 3).

In brain, there was no significant change in the levels of MDA and endogenous antioxidants (SOD, CAT and GSH) in animals exposed to lead alone or in combination with *Spirulina* when compared to control.

DISCUSSION

In the development of diseases, the toxic metabolites (free radicals) have emerged as a major entity that encourage the damage to the cells. This is because many constituents of the cell are the potential substrates of free radical attack. These toxic metabolites are generated by aerobic metabolism in the cell, which in turn significantly increase the pathological conditions. A frequent cellular target is lipid component, free radical mediated denaturation of proteins, enzymatic deactivation, base hydroxylation of

nucleic acids, cross linking or strand scission, mutation or even cell death. The extracellular components, including hyaluronic acid and collagen are also vulnerable to tissue injury by toxic oxidants. In these cases, the administration of exogenous antioxidants to counteract the proportionate magnitude of the cell injury plays a pivotal role in the treatment of free radical mediated injury or disease.

This present study reveals that, *Spirulina* showed a protective effect against lead induced alteration in the levels of MDA and endogenous antioxidants in the liver, lung, heart and kidney of rats. Cell death has been reported in the lead intoxicated animals because of acceleration of iron dependent lipid peroxidation (Quinlan et al., 1988). There is a generation of free oxygen radicals and δ-amino laevulinic acid during the lead exposure. These peroxides have been shown to cause cellular damage (Monterio et al., 1989). The peroxides are capable of changing membrane structure restricting phospholipid movement and facilitate the propagation of peroxides (Quinlan et al., 1988). However, *Spirulina* is a very rich source of antioxidants such as β-carotene and SOD enzyme. This study indirectly evidences that, there is a free radical induction after chronic low level exposure to lead in the organs like liver, lung, heart and kidney of rats. Lead is reported to stimulate the release the free radical like hydroxyl radical (Ding, et al., 2000). Increase in the levels of MDA suggests that there is stimulation in the process of lipid peroxidation in organs like liver, lung, heart and kidney of rats after lead exposure.

SOD metabolizes superoxide radical anion. It is an effective defense of the cell against endogenous and exogenous generation of superoxide radical (Brawn and Fridovich, 1980). In this present study, the SOD levels in liver, lung, heart and kidney were decreased in the lead intoxicated animals. However, in *Spirulina* treated animals the SOD levels were restored to normal even during lead exposure. CAT has been reported to be responsible for detoxification of significant amount of hydrogen peroxide (Brenner and Alison, 1953; Nicholls, 1965). CAT may function to protect the cells against onslaught of horrendous amount of hydrogen peroxide. CAT deficient organisms are more rapidly killed by hydrogen peroxide (MamEation, 1990). The SOD, CAT and GSH levels were reported to decrease in rats exposed to lead with an increase in the lipid peroxidation (El Missary, 2000). In the present study the CAT levels in the liver, lung, heart and kidney were found to decreased in animals on lead exposure. Co-administration of *Spirulina* restored the CAT levels to normal in organs of rats. Reduced glutathione (GSH) is a protective molecule against chemical induced cytotoxicity (Orrenius and Moldeus, 1984). GSH metabolism plays a vital role in many biological processes; in detoxification of xenobiotics, reactions of oxygen free radicals (Meister, 1984). GSH depletion and/or oxidative stress were responsible for changes in the expression of some or all glutathione-s-transferases that follow lead exposure. The lead exposure has been found to decrease the GSH levels in animals (Gurer et al., 1998). Similar results were also found in the present study that, the levels of GSH in liver, lung, heart and kidney were decreased after lead exposure. However, simultaneous administration of *Spirulina* did not result in significant decrease of GSH levels in the organs. This indicates a protective antioxidant effect of *Spirulina*. The modulatory effect of *Spirulina* on lead toxicity may be attributed to the presence of the antioxidant β-

carotene and SOD enzyme (Shastri et al., 1999).

Inspite of higher deposition of lead in brain of group II and group IV animals there were no significant changes in the levels of LPO and endogenous antioxidants conforming the earlier findings (Gelman and Michaelson, 1979; Gelman et al. 1979).

It has been long known that dietary calcium deficiency enhances lead absorption (Six and Goyer, 1970). It is also known that vulnerable site for localization of lead is the calcified matrix of the skeleton. But the major concern has been with the disposition of lead in the other more toxicologically significant tissues, e.g. liver, lung, heart, kidney, brain and hemopoietic system. Following administration or exposure in rats, the concentration of lead in soft tissues falls rapidly, largely as a result of transfer to the skeleton. An approximate steady state with regard to inter-compartmental distribution is attained in about 14 days (Hammond, 1971). The pattern of distribution is independent of dose over a wide range and is quite similar for rats and rabbits (Hammond, 1971). In this present study, considerably large amount of lead deposition was found in the liver, lung heart, kidney and brain of rats. Further, it has been also noted that, *Spirulina* did not have any inhibitory action on absorption or distribution of lead in liver, lung, heart and kidney. Interestingly, in present study, there was a significant decrease in the deposition of lead in group IV animals suggesting *Spirulina* protecting the brain from lead deposition. The exact action and mechanism by which this protection is taking place will be the new area of research.

The deleterious effects of lead on the central nervous system may range from behavioral dysfunction to encephalopathy, particularly in developing animals. Lead induced heperactivity has been reported in mice (Silbergeld and Goldberg, 1973), and in monkeys (Allen et al., 1974). Lead was given in drinking water or food to nursing mothers or directly to pups immediately after birth and before weaning. These animals exhibited significant increase in motor activity and poor learning performance. In such hyperactive mice the increased motor activity was suppressed by the administration of amphetamine, methyl phenidate, cholinergic agonists and aminergic antagonists (Silbergeld and Goldberg, 1975). This concept is not universally accepted because of lack of reproducibility in rats (Sobotka and Cook, 1974; Mano et al., 1980). In this present study it has been found that, chronic low level lead exposure (100ppm) has not shown any behavioral change in the experimental animals. Further, there was no significant change in the levels of MDA and endogenous antioxidants (SOD, CAT and GSH) in brains of animals exposed to lead or in combination with *Spirulina*.

The present study suggested that, *Spirulina fusiformis* prevents lipid peroxidation and restores the levels of endogenous antioxidants to normal in liver, lung, heart and kidney of lead exposed animals. Further, lead exposure at 100 ppm concentration has not changed the LPO or endogenous antioxidant levels in brain. However, it is interesting to note that *Spirulina* treatment prevents the deposition of lead in the brain.

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Table 1: Effect of lead exposure (30 days) alone and in combination with *Spirulina* (1500mg/kg) on the levels of lipid peroxidation or malondialdehyde formation in the organs of rats
[nM of MDA/mg protein]

Group	Liver	Lung	Heart	Kidney	Brain
I. Control	0.886 ± 0.019	0.585 ± 0.010	0.295 ± 0.007	1.031 ± 0.049	1.254 ± 0.040
II.100ppm Lead Acetate (LA)	1.856 ± 0.020[a]	0.989 ± 0.010[a]	0.541 ± 0.010[a]	1.482 ± 0.021[a]	1.371 ± 0.029
III. *Spirulina* (SP) (1500mg/kg)	0.827 ± 0.019	0.493 ± 0.008	0.285 ± 0.018	0.949 ± 0.053	1.184 ± 0.021
IV. 100ppm LA+SP (1500mg/kg)	1.058 ± 0.036[b]	0.692 ± 0.012[b]	0.331 ± 0.014[b]	1.100 ± 0.017[b]	1.305 ± 0.023

Values expressed as Mean ± SEM (n=6) for each observation
[a]$p<0.001$ When group II is compared with group I
[b]$p<0.001$ When group IV is compared with group II

Table 2: Effect of lead exposure (30 days) alone and in combination with *Spirulina* (1500mg/kg) on the levels of endogenous antioxidants in the organs of rats

Group	Liver	Lung	Heart	Kidney	Brain
Superoxide dismutase (U/mg protein)					
I. Control	7.753 ± 0.516	8.688 ± 0.050	7.212 ± 0.133	9.380 ± 0.101	10.321 ± 0.466
II.100ppm Lead Acetate (LA)	5.278 ± 0.064[a]	5.960 ± 0.075[a]	4.798 ± 0.163[a]	5.425 ± 0.101[a]	8.849 ± 0.822
III. *Spirulina* (SP) (1500mg/kg)	8.123 ± 0.320	8.861 ± 0.138	7.733 ± 0.205	9.578 ± 0.123	11.960 ± 0.848
IV. 100ppm LA+SP (1500mg/kg)	6.767 ± 0.134[b]	8.340 ± 0.058[b]	6.744 ± 0.120[b]	7.979 ± 0.291[b]	9.246 ± 0.763
Catalase (Kat f)					
I. Control	148.716 ± 1.663	117.902 ± 2.342	87.716 ± 1.476	116.849 ± 2.753	148.163 ± 7.394
II.100ppm Lead Acetate (LA)	56.013 ± 1.185[a]	65.050 ± 1.235[a]	40.367 ± 1.089[a]	77.497 ± 0.675[a]	132.012 ± 7.086
III. *Spirulina* (SP) (1500mg/kg)	159.462 ± 3.336	134.050 ± 3.113	94.561 ± 5.741	125.921 ± 2.156	149.321 ± 7.027
IV. 100ppm LA+SP (1500mg/kg)	113.393 ± 1.781[b]	109.592 ± 2.071[b]	81.361 ± 0.787[b]	106.331 ± 1.268[b]	147.181 ± 8.545
Reduced glutahthione (μM/mg protein)					
I. Control	9.971 ± 0.177	4.070 ± 0.125	4.073 ± 0.138	5.881 ± 0.098	5.188 ± 0.290
II.100ppm Lead Acetate (LA)	2.570 ± 0.243[a]	2.064 ± 0.127[a]	2.212 ± 0.065[a]	2.159 ± 0.163[a]	4.706 ± 0.402
III. *Spirulina* (SP) (1500mg/kg)	10.270 ± 0.221	4.272 ± 0.139	4.213 ± 0.110	6.057 ± 0.092	5.453 ± 0.264
IV. 100ppm LA+SP (1500mg/kg)	6.983 ± 0.117[b]	3.585 ± 0.034[b]	3.381 ± 0052[b]	5.316 ± 0.076[b]	4.928 ± 0.485

Values expressed as Mean ± SEM (n=6) for each observation
[a]p<0.001 When group II is compared with group I
[b]p<0.001 When group IV is compared with group II

Table 3: Tissue levels of lead in different experimental groups
[mg/g of tissue]

Group	Liver	Lung	Heart	Kidney	Brain
I. Control	0.0156 ± 0.0204	0.0247 ± 0.0051	0.0161 ± 0.0069	0.0164 ± 0.0079	0.011 ± 0.001
II.100ppm Lead Acetate (LA)	0.0841 ± 0.0344[a]	0.0114 ± 0.0245[a]	0.0719 ± 0.0215[a]	0.0103 ± 0.0476[a]	0.051 ± 0.009[b]
III. *Spirulina* (SP) (1500mg/kg)	0.0148 ± 0.0109	0.0237 ± 0.0195	0.0143 ± 0.0085	0.0140 ± 0.0042	0.010 ± 0.001
IV. 100ppm LA+SP (1500mg/kg)	0.0751 ± 0.0279	0.0108 ± 0.0387	0.0666 ± 0.0392	0.0882 ± 0.0412	0.030[c] ± 0.001

Values expressed as Mean ± SEM (n=6) for each observation
[a]$p<0.001$ When group II is compared with group I
[b]$p<0.01$ When group II is compared with group I
[c]$p<0.05$ When group IV is compared with group II

Indian Journal of Pharmacology 2001; 33: 185-191 RESEARCH PAPER

EFFECT OF VITAMIN E, VITAMIN C AND SPIRULINA ON THE LEVELS OF MEMBRANE BOUND ENZYMES AND LIPIDS IN SOME ORGANS OF RATS EXPOSEDTO LEAD

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SUMMARY

Objectives: To study the effect of lead alone and its combination with vitamin E, vitamin C and spirulina on the levels of membrane bound enzymes and lipids in some organs of rats.

Methods: Lead acetate (100 ppm) alone and its combinations with vitamin E, vitamin C or spirulina were fed to the rats for thirty days. Na^+-K^+-ATPase, Ca^{++}-ATPase, Mg^{++}-ATPase were estimated in liver and kidney of rats. Similarly the tissue lipids (Cholesterol, Triglycerides and Phospholipids) were also measured in the liver, lung, heart and kidney of rats.

Results: Lead acetate significantly ($p<0.001$) inhibited the levels of membrane bound enzymes in the liver and kidney of rats. Further, there was a significant ($p<0.001$) increase in the levels of cholesterol, triglycende and phospholipid in the liver, lung, heart and kidney of animals exposed to lead. Simultaneous administration of vitamin E (50 IU/kg), vitamin C (800 mg/kg) or spirulina (1500 mg/kg) along with lead restored the levels of membrane bound enzymes as well as the lipids in the animal tissues to normal levels

Conclusion: It is concluded that vitamin E, C or spirulina had a significant antioxidant activity thereby protecting the organs from the lead-induced toxicity.

KEYWORDS Lead vitamin E vitamin C spirulina ATPases lipids

INTRODUCTION

Exposure to various environmental contaminants such as lead produces overt clinical manifestations. Chronic low-level exposure of lead is known to produce varieties of toxicities in human being[1-4]. Lead is also reported to reduce the levels of cytochrome P-450[5] and cause other biochemical toxicities. Absorption of inorganic lead is known for its biochemical and metabolic toxicity[6]. Further, lead is also reported to release free radicals (hydroxyl)[7] thereby stimulating the process of lipid peroxidation[8]. Lipid peroxides have been shown to impair tissue membranes, which is a risk factor in varieties of diseases. Lead is reported to have an inhibitory action on the membrane bound[9] enzymes such as Na^+-K^+-ATPase, Ca^{++}-ATPase and Mg^{++}-ATPase in various vital organs. Studies[10] also have demonstrated that the lead exposure may modify the metabolism of lipids, decrease in the plasma cholesterol, HDL cholesterol fractions.

Recently, it was shown that Spirulina fusiformis, (SP) popularly known as the Blue Green Algae (Cyanobacterium), the most powerful food on earth, is rich in all the three types of micronutrients, proteins, lipids and carbohydrates and some more vital elements like zinc, magnesium, manganese, selenium andvitamins like ,β-carotene, riboflavin cyanocobolamin, α-tocopherol and α-linoleic acids[11]. Besides, Spirulina is also known to have a protective antioxidant effect. The effect of Spirulina on the lead induced changes on lipids and membrane bound enzymes, which is a consequence of oxidative stress have not been studied yet. Therefore, the present work is aimed at studying the effect of Spirulina along with the known antioxidants like vitamin E[12], C[13] on

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the levels of tissue membrane bound enzymes like Na^+-K^+-ATPase, Ca^{++}-ATPase and Mg^{++}-ATPase (ATP phosphohydrolase EC 3.6.1.3.) and various lipids in vital organs like liver, lung, heart and kidney of rats exposed to lead.

MATERIALS AND METHODS

Spirulina was obtained commercially from Indon Healthcare Ltd. Aslali, Ahmedabad. α-tocopherol acetate, Tris-hydrochloride buffer, Adenosine mono and tri phosphate, ferric chloride hexahydrate, triolein were purchased from Hi-Media Laboratories Pvt. Ltd. Bombay. Cholesterol, trichloroacetic acid, ammonium molybdate, acetylacetone, perchloric acid were obtained commercially from SD Fine Chemicals, Bhoisar, Bombay. Other routine chemicals were obtained commercially from the above mentioned companies. All reagents of analytical grade were used in the entire study.

Adult albino rats (Wistar strain) of either sex weighing 150-225g were maintained at 25 ± 3°C in a well-ventilated animal house under natural photoperiod conditions in large polypropylene cages. The animals were divided into eight groups of six in each group and were given the following treatment:

Group I (Control group): Double Deionised Water (DDW) and normal food.

Group II (LA): Lead acetate 100 ppm in DDW (100 ppm LA).

Group III (E): Vitamin E (50 IU/kg) mixed with food and DDW.

Group IV (E+LA): Vitamin E (50 IU/kg) mixed with food and 100 ppm LA in DDW.

Group V (C): Vitamin C (800 mg/kg) mixed with food and DDW.

Group VI (C+LA): Vitamin C (800 mg/kg) mixed with food and 100 ppm LA in DDW.

Group VII (SP): Spirulina (1500 mg/kg) mixed with food and DDW.

Group VIII (SP+LA): Spirulina (1500 mg/kg) and 100 ppm of LA in DDW.

Lead in the form of lead acetate (100 ppm) was dissolved in DDW as reported earlier[14] and was given *ad libitum*. Vitamin E in the form of α-tocopherol acetate (50 IU/kg), vitamin C as ascorbic acid (800 mg/kg) or Spirulina (1500 mg/kg) were mixed with food. The food consisted of untreated seed rye flour (60%), powdered skimmed milk (30%), corn oil (9%), sodium chloride (1%) with added vitamins and iron. All the animals were treated for a period of thirty days as described above.

At the end of thirty days, rats were deprived of food over night and all the animals were sacrificed by carotid bleeding after being anaesthetized by urethane (120 mg/100 gm, *i.p*). Liver and kidney were collected in cold conditions by using pre-cooled petri dishes and were blotted free of blood and tissue fluid. After being weighed the organs were cross-chopped with surgical scalpel into fine slices, chilled in the ice-cold 0.25 M sucrose and then blotted on a filter paper. The tissues were minced and homogenized in 10 mM Tris-HCl buffer (pH-7.4) at a concentration of 10%w/v with 25 strokes of tight Teflon homogeniser at a speed of 2500 rpm. The prolonged homogenization under hypotonic condition disrupted the ventricular structure of cells so as to release soluble proteins and leave membrane and non-vascular matter in a sedimental form. The homogenates were centrifuged at 10,000 X g at 4°C for 20 minutes[15]. The supernatant was recentrifuged for 1 hour at 100000 X g at 4°C using Remi C-24 high speed cooling centrifuge. The sediment was resuspended in Tris-HCl buffer (pH 7.4) to get the final concentration 10% and was used for the assay of membrane bound enzymes. The enzymes sodium potassium ATPase[16], calcium ATPase[17], magnesium ATPase[18] and proteins[19] were estimated in the liver and kidney of rats using 10% membrane homogenates.

Tissue lipids were extracted[20] from the organs like liver, lung, heart and kidney and were subjected to the estimation of cholesterol[21], triglycerides[22] and phospholipids[23] were estimated in liver, lung, heart and kidney of rats using extracted lipid layers.

Statistical analysis: The mean ± SEM values were calculated for each group to determine the significance of intergroup difference. Each parameter was analyzed separately using one way analysis of variance (ANOVA) with Huynh-Feldt epsilon modification of degrees of freedom to correct the departure from sphericity. To find the difference between the groups Student ' *t* ' test was used. P values <0.05 were considered to be significant.

RESULTS

The membrane bound Na^+-K^+-ATPase, Ca^{++}-ATPase and Mg^{++}-ATPase levels on liver and kidney were significantly ($p<0.001$) decreased in lead exposed (LA) animals as compared to control (Table 1). It was further shown that there was a significant ($p<0.001$) increase in levels of cholesterol, triglyceride and phospholipid in liver, lung, heart and kidney in lead exposed (LA) group as compared to control (Table 2).

Vitamin E treatment (E+LA) to the lead exposed animals showed a significant ($p<0.001$) increase in the levels of Na^+-K^+-ATPase, Ca^{++}-ATPase and Mg^{++}-ATPase in liver and kidney as compared to the animals exposed to lead alone (LA) (Table 1). These results indicate a protective effect of vitamin E on the membrane bound ATPase activity by virtue of its antioxidant property. Treatment with vitamin E to lead (E+LA) exposed animals, showed a significant ($p<0.001$) decrease in the levels of cholesterol, triglycerides and phospholipids in the liver, lung, heart and kidney as compared animals exposed to lead alone (LA) (Table 2). These results show that, vitamin E has a protective effect on the lead induced changes in the lipid levels.

In liver, vitamin C treatment to lead (C+LA) exposed animals, showed a significant increase in the levels of Na^+-K^+-ATPase ($p<0.05$), Ca^{++}-ATPase ($p<0.001$) as compared to the animals exposed to lead alone (LA). In kidney, vitamin C treatment in the lead exposed animals showed a significant increase in the levels of Na^+-K^+-ATPase ($p<0.001$), Ca^{++}-ATPase ($p<0.01$) and Mg^{++}-ATPase ($p<0.001$) as compared to lead exposed animals alone (LA) (Table 1). Vitamin C treatment to animals exposed to lead (C+LA) showed a significant decrease in the levels of cholesterol ($p<0.001$), triglycerides ($p<0.05$) and phospholipids ($p<0.001$) in the liver, lung, heart and kidney as compared to group II (LA) (Table 2).

Spirulina treatment to lead (SP+LA) exposed animals, showed a significant ($p<0.001$) increase in the in the levels of Na^+-K^+-ATPase, Ca^{++}-ATPase and Mg^{++}-ATPase in liver and kidney as compared to the animals exposed to lead alone (LA) (Table 1) implying a protective effect of Spirulina on the membrane bound enzymes of liver and kidney. Spirulina treatment to lead (SP+LA) exposed animals, showed a significant ($p<0.001$) decrease in the levels of cholesterol,

Table 1. Effect of lead acetate (LA) treatment (30 days) alone and in combination with vitamin E (50 IU/kg), vitamin C (800 mg/kg) or Spirulina (1500 mg/kg) on the levels of membrane bound enzymes in liver and kidney of rats. (Unit expressed as mM of Pi liberated/mg proteins)

	Liver	Kidney
Na^+-K^+ ATPase		
I. Control	2.297 ± 0.097	3.545 ± 0.025
II. 100ppm (LA)	0.672 ± 0 012***	1.838 ± 0.027***
III. Vitamin E (E)	2.323 ± 0.017	3.568 ± 0.103
IV. Vitamin E + LA	1.863 ± 0 009***	2.561 ± 0.064***
V. Vitamin C (C)	2.300 ± 0.022	3.564 ± 0.131
VI. Vitamin C + LA	1.092 ± 0.133*	2.444 ± 0 062***
VII. Spirulina (SP)	2.368 ± 0.053	3.784 ± 0.166
VIII. Spirulina + LA	2.095 ± 0.016***	2.996 ± 0.018***
ANOVA. P<	0.001	0.001
Ca^{++}-ATPase		
I. Control	1.370 ± 0.095	1.797 ± 0.010
II. 100ppm (LA)	0.472 ± 0.011***	0.992 ± 0 021***
III. Vitamin E (E)	1.378 ± 0.038	1.800 ± 0.029
IV. Vitamin E + LA	0.826 ± 0.026***	1.388 ± 0.023***
V. Vitamin C (C)	1.371 ± 0.029	1.799 ± 0.028
VI. Vitamin C + LA	0.749 ± 0.011***	1.156 ± 0 037**
VII. Spirulina (SP)	1.403 ± 0.069	1.809 ± 0.035
VIII. Spirulina + LA	1.012 ± 0.014***	1.526 ± 0.014***
ANOVA: P<	0.001	0.001
Mg^{++}-ATPase		
I. Control	1.552 ± 0.014	2.880 ± 0.104
II. 100ppm (LA)	0.985 ± 0.018***	1.040 ± 0.023***
III Vitamin E (E)	1.583 ± 0.017	2.927 ± 0.031
IV. Vitamin E + LA	1.259 ± 0.010***	2.279 ± 0.040***
V Vitamin C (C)	1.571 ± 0.065	2.882 ± 0.056
VI. Vitamin C + LA	1 047 ± 0.063	2.126 ± 0.121***
VII. Spirulina (SP)	1.597 ± 0.079	3.070 ± 0.057
VIII. Spirulina + LA	1.326 ± 0.041***	2.716 ± 0.020***
ANOVA: P<	0.001	0.001

The values expressed as mean ± SEM (n=6).
Group II (LA) is compared with group I(Control).
Group IV (E+LA), group VI (C+LA) and group VIII (SP+LA) are compared with group II (LA).
Group III (E), group V (C) and group VII (SP) are compared with group I (Control).

*** P<0.001, ** P<0.01, * P<0.05 (Student's '*t*' test)

Table 2. Effect of lead acetate (LA) treatment (30 days) alone and in combination with vitamin E (50 IU/kg), vitamin C (800 mg/kg) or Spirulina (1500 mg/kg) on the levels of lipids in liver, lung, heart and kidney of rats. (mg/gm of wet fresh tissue)

	Liver	Lung	Heart	Kidney
Cholesterol				
I Control	6 373 ± 0.085	4 444 ± 0 013	2 959 ± 0.108	5.545 ± 0.031
II 100 ppm (LA)	12.095 ± 0 179***	9.693 ± 0 166***	6.809 ± 0.213***	9.751 ± 0.150***
III Vitamin E (E)	6.026 ± 0.116	4.168 ± 0 079	2.807 ± 0.084	5 191 ± 0 092
IV Vitamin E + LA	7 987 ± 0.340***	6.803 ± 0.281***	3.717 ± 0 212***	6.805 ± 0 280***
V Vitamin C (C)	6 364 ± 0 103	4.398 ± 0.034	2 776 ± 0.136	5.533 ± 0.046
VI Vitamin C + LA	8.846 ± 0 393***	6.576 ± 0.105***	4.739 ± 0.098***	6.526 ± 0.107***
VII. Spirulina (SP)	6.179 ± 0 013	3.866 ± 0 177	2.770 ± 0.030	5 252 ± 0.100
VIII Spirulina + LA	7 131 ± 0 068***	5 307 ± 0.060***	3.370 ± 0.112***	6.275 ± 0.038***
ANOVA: P<	0.001	0.001	0.001	0.001
Triglycerides				
I Control	9.232 ± 0.097	3 482 ± 0 152	2.963 ± 0.078	7.185 ± 0 114
II 100 ppm (LA)	16.689 ± 0.191***	9.678 ± 0.109***	6.622 ± 0.091***	11.386 ± 100***
III Vitamin E (E)	9 267 ± 0 125	3 626 ± 0.128	2.958 ± 0.296	7.256 ± 0.121
IV Vitamin E + LA	11 937 ± 0.141***	5 739 ± 0.084***	4 902 ± 0.149***	9.169 ± 0.105***
V Vitamin C (C)	9.264 ± 0 328	3.497 ± 0 120	2 980 ± 0.258	7 208 ± 0 258
VI Vitamin C + LA	15.762 ± 0 317*	8.447 ± 0 527*	5.623 ± 0 354*	9.975 ± 0 439*
VII. Spirulina (SP)	9.253 ± 0.166	3 502 ± 0.125	2.967 ± 0 283	7.193 ± 0.102
VIII. Spirulina + LA	11 672 ± 0 191***	4.218 ± 0 145***	3.499 ± 0 299***	8 093 ± 0 217***
ANOVA. P<	0.001	0.001	0.001	0.05
Phospholipids				
I. Control	9.141 ± 0 136	3.516 ± 0 047	2.515 ± 0.047	3.397 ± 0.079
II. 100 ppm (LA)	20 453 ± 0.197***	9 488 ± 0 060***	8.488 ± 0 060***	11.750 ± 0.356***
III Vitamin E (E)	9.154 ± 0.245	3.563 ± 0 084	2.546 ± 0.093	3.406 ± 0.074
IV Vitamin E + LA	10 471 ± 0 132***	4.805 ± 0 297***	4.135 ± 0.120***	6.506 ± 0.135***
V Vitamin C (C)	9.173 ± 0.221	3.564 ± 0.188	2 547 ± 0.153	3 409 ± 0.065
VI Vitamin C + LA	10.527 ± 0 062***	6.429 ± 0.332***	5.996 ± 0.051***	7 274 ± 0 130***
VII. Spirulina (SP)	9.146 ± 0.087	3.479 ± 0.084	2.327 ± 0.120	3 378 ± 0 079
VIII Spirulina + LA	10.112 ± 0.069***	4.261 ± 0.189***	3 261 ± 0.261***	5.996 ± 0 051***
ANOVA. P<	0 001	0.001	0.001	0.001

The values expressed as mean ± SEM (n=6) Group II (LA) is compared with group I (Control). Group IV (E+LA), group VI (C+LA) and group VIII (SP+LA) are compared with group II (LA). Group III (E), group V (C) and group VII (SP) are compared with group I (Control). *** P<0 001, ** P<0.01, * P<0.05 (Student's '*t*' test)

triglycerides and phospholipids in the liver, lung, heart and kidney as compared to animals exposed to lead alone (LA) (Table 2).

DISCUSSION

In varieties of disease development processes, the toxic metabolites have emerged an as major final common entity that encourages the damage. This is because many constituents of the cell are the potential substrates of free radical attacks[24]. These toxic metabolites are generated by aerobic metabolism in the cell, which in turn significantly increase the pathological conditions. A frequent cellular target is lipid component, free radical mediated denaturation of proteins, enzymatic deactivation, base hydroxylation of

nucleic acids, cross linking or strand scission, mutation or even in cell death. The extracellular components, including hyaluronic acid and collagen are also vulnerable to tissue injury by toxic oxidants. In these cases, the administration of exogenous antioxidants to counteract the proportionate magnitude of the cell injury plays a pivotal role in the treatment of free radical mediated injury or disease.

The present experiment demonstrated that there is a free radical induction after chronic low level exposure to lead in the organs like liver, lung and kidney of the rats. It has been reported earlier that, heavy metals are involved in varieties of disorders like hypertension[25], renal and hepatic disorders[26]. Lead is a toxic metal and it is present in various ecosystems like soil, air and water, which are the fundamental necessities of animal and humans.

Lead is reported to stimulate the release of free radicals like hydroxyl radical. Lead alters the cellular structures like membrane lipids and cellular functions like detoxification processes, removal of hydroxyperoxides, protection against effect of ionizing radiations, cellular growth. It is further reported that lead modulates many enzymes, disulfhydril status of proteins[27].

The lipid dependent membrane bound enzymes are ATPase. Any alteration in membrane lipid leads to change in membrane fluidity, which in turn alters the ATPase activities and cellular functions. A certain degree of membrane fluidity seems to be essential for Na^+-K^+ATPase. The fluidity of the membrane, to a large extent, is determined by the fatty acids[28]. Increased enzyme activity was reported with changes in the levels of membrane cholesterol and phospholipids[29].

In present study levels of Na^+-K^+ATPase, Ca^{++}-ATPase and Mg^{++}-ATPase were reduced in liver and kidney of lead treated animals. This may be because of the changes in the levels of cholesterol, phospholipids and triglycerides. As the levels of ATPase are more likely to be change with the change in the levels of these lipids. Lipid peroxidation is a complex and natural deleterious process. The decrease in the levels Na^+-K^+ATPase, Ca^{++}-ATPase and Mg^{++}-ATPase could be due to enhanced lipid peroxidation by free radicals in lead treated animals. Since these membrane bound enzymes are 'SH' group containing enzymes, which are lipid dependent[30]. In the liver and kidney of vitamin E, vitamin C or spirulina treated animals, the levels of Na^+-K^+ATPase, CA^{++}-ATPase and Mg^{++}-ATPase has been restored to near normal. The restored activities of ATPases suggests the ability of vitamin E, vitamin C or spirulina to protect the sulfhydryl group from oxidative damage through inhibition of peroxidation of membrane lipids in liver and kidney or rats.

Lipids are the important constituents of the organs. Inorganic lead is a pro-oxidant and peroxidative damage to cellular membrane lipids and fatty acids leads to membrane fragility and permeability is a likely consequence of lead poisoning. The present study demonstrate the significant increase in the levels of cholesterol, triglycerides and phospholipids in the liver, lung, heart and kidney of rats treated with lead. This may be attributed to the ability of lead to alter the cellular structures. Other reason for increase in the levels of lipids may be increase in the lipid peroxidative processes by releasing free radicals. It has been reported[31] that, a single dose administration of lead induces cellular proliferation in the organs like liver and kidney. Similarly, the increase in the levels of triglycerides in lead treated animals indicating the breakdown of fatty acids. As reported[8] earlier that fatty acid decomposition increases in lead treated animals. Lead is reported[32] to cause consistent increase in the vagal activity including bradycardia and other cardiac disorders. High levels of lipids are also seen in the kidney of lead treated animals.

It is well known that vitamin E, vitamin C are the well-known antioxidant vitamins which protects the cell from free radical attack. In the present study vitamin E, vitamin C has been shown to normalize the levels of ATPase and the lipids in the various organs of the experimental animals.

The blue-green algae (Cyanobacterium) Spirulina has been used both as a dietary supplement and as a medicinal substance. In Spirulina supplemented (10-30%) diet, the rat did not show any abnormalities in organ weight of the liver, lung, kidney, heart and spleen. Spirulina fed rats showed 3 folds increase in lactobacillus content and a 43% increase in vitamin B1 in the caecum of rats. Rats fed on Spirulina have reduced kidney toxicity from mercury poisoning[33]. Spirulina is rich in β-carotene and the bioavailability is as good as the pure β-carotene.

It has been suggested[34] that the Spirulina extracts could be effective against free radical induced lipid peroxidation which in turn may lead to cellular transformation. In present study Spirulina has shown protective activity on the liver, lung, heart and kidney of the experimental animals. It was also found to have better protective property than the vitamin C.

It is concluded that, chronic low level exposure to lead resulted in decrease in the levels of ATPase and increase in the levels of lipids. This may be due to the release of free radicals by lead. Simultaneous treatment with vitamin E, vitamin C or Spirulina in rats exposed to lead resulted in the reversal of the levels of ATPase in liver and kidney of rats. The antioxidants (vitamin E, C or Spirulina) have shown to restore the levels of lipids in the liver, lung, heart and kidney of rats exposed to lead acetate. Therefore, vitamin E, vitamin C and Spirulina may have a protective antioxidant effect against any injury to the organ caused by lead.

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Effect of lead with vitamin E, C, or *Spirulina* on malondialdehyde, conjugated dienes and hydroperoxides in rats

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Lead (100 ppm) was given in doubly deionised water for 30 days to one group of rats The other groups received lead along with exogenous antioxidants like vitamin E (50 IU/kg), vitamin C (800 mg/kg) or *Spirulina* (1500 mg/kg) in food for a similar period. Levels of lipid peroxidation products such as malondialdehyde, conjugated diene and hydroperoxide were measured in liver, lung and kidney of treated rats. In lead treated animals there was a significant increase in the levels of these lipid peroxidative products. Administration of exogenous antioxidants in the lead treated animals reduced the levels of malondialdehyde, conjugated diene and hydroperoxide. It indicated that vitamin E, vitamin C and *Spirulina* had significant (P<0 001) antioxidant activity thereby protecting the animals from lead induced toxicity.

Lead is one of the common toxic metals present in our environment. Clinical studies have shown that there may be an association between increased exposure of this metal and some behavioral and CNS disorders[1]. It is also been reported that lead has toxic effects on cardiovascular system[2] blood[3-4], kidney[5], and enzymes (biochemical toxicities)[6].

Lead is well known for its involvement in various biochemical and metabolic processes. Absorption of inorganic lead can lead to certain biochemical and metabolic toxicities[7-8]. Cause of lead toxicity may be its impact on the free radical release like hydroxyl radical[9]. Therefore, a need for reliable antioxidant arises, which must be capable of scavenging the free radicals when system is exposed to lead. Free radical attack is indicated by change in the levels of some biomolecules in the body. The process is known as lipid peroxidation (LPO). The main products of lipid peroxidation are malondialdehyde (MDA), conjugated dienes (Conj. dienes), hydroperoxides (HYPDX). The levels of these products increase during the free radical attack.

Primary objective of the study is to explore the effect of lead on the levels of malondialdehyde, conjugated dienes and hydroperoxides in the vital organs like liver, lung and kidney of rats. It is further aimed to find out the scope for exogenous antioxidants namely *Spirulina* as well as known antioxidants like vitamin E and vitamin C on free radical release by lead exposure in liver, lung and kidney of rats.

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Cyanobacterium, *Spirulina fusiformis* (SP) popularly known as the Blue Green Algae, the most powerful food on the earth, is rich in all the three types of micronutrients proteins, lipids and carbohydrates. Besides these micronutrients, some more vital elements like zinc, magnesium, manganese, selenium, some vitamins like β-carotene, riboflavin, cyanocobalamin, α-tocopherol, α-linoleic acids are also present[10].

Spirulina was obtained commercially from Indon Healthcare Ltd., Aslali, Ahmedabad. Cumene hydroperoxide, octadienoic acid conjugated methyl ester and 1,1,3,3-tetraethoxy propane were obtained from Sigma Chemicals, St. Louis, M.O., USA. α-tocopherol acetate, tris-hydrochloride buffer, thiobarbituric acid, cadmium acetate, sucrose were purchased from HiMedia Laboratories Pvt. Ltd., Bombay. Lead acetate, ascorbic acid, trichloroacetic acid, potassium iodide, chloroform, methanol, acetic acid, cyclohexane were obtained commercially from SD fine chemicals, Bombay. All reagents used were of analytical grade.

Adult albino rats (Wistar strain) of either sex weighing 150-225 g were maintained at 25°±3°C in well-ventilated animal house under natural photoperiod conditions. The animals were divided into eight groups of six in each group. Group I (Control), received doubly deionised water (DDW) and normal food. Group II (LA), rats were fed lead

acetate 100 ppm in DDW (100ppm LA). Group III (E), rats were fed vitamin E (50 IU/kg) mixed with food and DDW. Group IV (E+LA), rats were fed vitamin E (50 IU/kg) mixed with food and 100 ppm LA in DDW. Group V (C), rats were fed vitamin C (800 mg/kg) mixed with food and DDW. Group VI (C+LA), rats were fed vitamin C (800 mg/kg) mixed with food and 100 ppm LA in DDW. Group VII (SP), rats were fed *Spirulina* (1500 mg/kg) mixed with food and DDW. Group VIII (SP+LA), rats were fed Spirulina (1500 mg/kg) mixed with food and 100 ppm LA in DDW.

Lead in the form of lead acetate (100 ppm) was dissolved in DDW[11] and was given *ad libitum*. Vitamin E in the form of α-tocopherol acetate (50 IU/kg), vitamin C as ascorbic acid (800 mg/kg) or SP (1500 mg/kg) were mixed with food. The food consisted of untreated seed rye flour (60%), powdered skimmed milk (30%), corn oil (9%), sodium chloride (1%) with added vitamins and iron. All the animals were treated for a period of thirty days as described above.

At the end of thirty days, rats were deprived of food over night and all the animals were sacrificed by carotid bleeding after being anaesthetized by urethane (120 mg/100g ip). Liver, lung and kidney were collected in cold conditions by using precooled petri dishes and were blotted free of blood and tissue fluid. After being weighed the organs were cross-chopped with surgical scalpel into fine slices, chilled in the ice-cold sucrose (0.25*M*) and then blotted on a filter paper. The tissues were minced and homogenized in 10m*M* tris-HCl buffer (*p*H 7.4) at a concentration of 10% w/v with 25 strokes of tight Teflon homogenizer at a speed of 2500 rpm. The prolonged homogenization under hypotonic condition disrupted the ventricular structure of cells so as to release soluble protein and leave membrane and non-vascular matter in a sedimental form. The homogenates were centrifuged at 10,000 *g* at 4°C for 20 min[12-13]. The supernatant was recentrifuged for 1hr at 10,0000 *g* at 4°C using Remi C-24 high-speed cooling centrifuge. The clear supernatant was used for the enzyme assays.

The tissue levels of malondialdehyde[14], conjugated diene[15], hydroperoxides[16] and tissue proteins[17] were estimated as mentioned earlier.

Statistical analysis—Data was subjected to statistical analysis. Each parameter was analyzed separately by using one way analysis of variance (ANOVA) with Huynh-Feldt epsilon modification of degree of freedom to correct the departure from sphericity. To find the difference between the groups Student's t test was used.

Effect of vitamin E, C or SP on tissue malondialdehyde (MDA)—MDA levels increased significantly in liver, lung and kidney of group II animals as compared to group I. MDA levels in liver and lung decreased significantly in group IV, group VI and in group VIII when compared with group II. In kidney, MDA levels decreased significantly in group IV, group VI and in group VIII when compared to group II. However, there was no significant change in the MDA levels in group III, group V and group VII when compared with group I (Table 1).

Effect of vitamin E, C or SP on tissue conjugated dienes (Conj. diene)—Tissue conjugated dienes levels increased significantly in liver, lung and kidney of group II as compared with group I. The conjugated diene levels in liver decreased significantly in group IV, group VI and group VIII when compared to group II. In lung and kidney the levels were decreased significantly in group IV, group VI and in group VIII when compared to group II. However, there was no significant change in the tissue conjugated diene levels in group III, group V and group VII when compared to group I (Table 1).

Effect of vitamin E, C or SP on tissue hydroperoxides (HYPDX)—HYPDX levels of liver, lung and kidney of group II as compared with group I. HYPDX levels in the liver decreased significantly in IV, in group VI, and in group VIII when compared to group II. HYPDX levels of lung and kidney decreased significantly in group IV and in group VIII when compared with group II. In group VI, HYPDX levels were insignificant in lungs and were significant in kidney. However, there was no significant change in HYPDX levels in group III, group V and group VII when compared to group I (Table 1).

Free radicals are involved in normal physiological processes in the living organisms. They act as the messenger for signal transduction and also affect the gene expression[18]. There are several proteins and biomolecules in the living organism, which act as free radical scavengers. Besides these biomolecules, several diet supplements containing vitamins, polyphenols, flavones also play a significant role in this matter[19]. As the concentration of these biomolecules remains optimum there is no physiological irregularities take place. When production of free radicals increases beyond the

Table 1 — Effect of lead acetate (LA) treatment (30 days) alone and in combination with vitamin E (50 IU/kg), vitamin C (800mg/kg) or *Spirulina* (1500 mg/kg) on malondialdehyde, conjugated diene and hydroperoxides in liver, lung and kidney of rats

[Values are Mean ± SE of 6 rats]

Group	Liver	Lung	Kidney
Malondialdehyde (m*M* of MDA formed/mg protein)			
I	0.886 ± 0 019	0 585 ± 0 010	1.031 ± 0.049
II	1 856 ± 0 020***	0.989 ± 0 010***	1.482 ± 0.021***
III	0 856 ± 0 007	0 528 ± 0 010	0 985 ± 0.016
IV	1.430 ± 0.042***	0.623 ± 0.054***	1.188 ± 0.012***
V	0.871 ± 0.011	0 578 ± 0.086	1.025 ± 0 022
VI	1 652 ± 0 026**	0 638 ± 0.085**	1.372 ± 0 005*
VII	0.827 ± 0.019	0 493 ± 0 008	0.949 ± 0.053
VIII	1.058 ± 0.036***	0 540 ± 0.012***	1.100 ± 0.017***
Conjugated dienes (m*M* of Conj. diene liberated/mg protein)			
I	64.018 ± 0.464	18.188 ± 0.208	16.822 ± 0 260
II	117.602 ± 0.422***	36.460 ± 0.305***	29 265 ± 0.417***
III	63.899 ± 0 268	17.777 ± 0.666	16.588 ± 0 313
IV	90.234 ± 0.484***	22.753 ± 0.197***	19 083 ± 0 206***
V	64 029 ± 0 614	17.940 ± 0 453	16.222 ± 1.002
VI	105 903 ± 4.529*	32 467 ± 1.660*	23.011 ± 0.345***
VII	63.126 ± 0.162	17.667 ± 0.175	16 527 ± 0 169
VIII	79.030 ± 0 519***	25 087 ± 0.239***	20.850 ± 0.233***
Hydroperoxides (n*M* of HYPDX liberated/mg protein)			
I	10.510 ± 0.084	13.074 ± 0.175	12.246 ± 0.096
II	20.022 ± 0 219***	28 924 ± 0 430***	19.660 ± 0.170***
III	10.403 ± 0.216	12.928 ± 0.128	12.192 ± 0.280
IV	12.843 ± 0 121***	16.078 ± 0.236***	13.939 ± 0.104***
V	10 482 ± 0.088	13 069 ± 0.193	12.229 ± 0.205
VI	18.778 ± 0.419*	25.838 ± 1.754	16 401 ± 0 388***
VII	10.258 ± 0.221	12.928 ± 0.114	12 523 ± 0.082
VIII	17 076 ± 0 611**	19.350 ± 0 283***	15.521 ± 0.075***

Group I-Control; II- 100ppm (LA); III- vitamin E; IV- vitamin E+LA; V-vitmain C; VI- vitamin C+LA; VII- *Spirulina*; and VIII- *Spirulina*+LA.
Significant at - ***P<0.001, **<0.01, *<0.05
Group II is compared with group I.
Group IV, group VI and group VIII are compared with group II.
Group III, group V and group VII are compared with group I.

normal; and these macromolecules and dietary components are not able to scavenge the raised levels of free radicals then the physiological or biochemical disorders or diseases occurred[20]. The family of bcl-2 gene expresses antioxidant proteins. Similarly phenols, flavonoids, alkaloids, terpenoids, organic acids and lipids act as natural free radical scavenger[21]. The present experiment demonstrated that, there is a free radical induction in the organs like liver, lung and kidney of rats after chronic low level exposure to lead. It has been reported earlier that, heavy metals are involved in varieties of disorders like hypertension[22], renal[23] and hepatic[24] disorders. Lead is a toxic metal and it is present in ecosystem like soil, air and water. Amongst these lead exposure through water is very common[25].

Lead is reported to stimulate the release of free radicals like hydroxyl radicals. Lead alters the cellular structures like membrane lipids and cellular functions like detoxification processes, removal of hydroperoxides etc. It is further reported that, lead modulates many enzymes, disulfhydryl status of proteins[26]. Lipid peroxidation is a free radical mediated process, which has been implicated in a variety of diseases. It is involved in the formation and propagation of lipid radicals, the uptake of oxygen, a