CHAPTER – 5

DISCUSSION

There was a significant decrease in the body weight of animals after thirty days exposure to 100 ppm of lead acetate and cadmium chloride as compared to control. However, no significant changes were observed in body weight of animals after thirty days exposure to 10 and 30 ppm of lead acetate and cadmium chloride. These results are in agreement with the earlier reports of Institoris et al. (1999) and Patra et al. (2001). It has been reported that lead and cadmium decreases the body weight of animals at higher dose levels, the probable reason being a dose dependent change in metabolic processes in body (Columbano et al., 1982; Choie and Richter, 1974; Patra et al., 2001; Goyer, 1995). Vitamin E and *spirulina* supplementation significantly increased the body weights of the normal animals but there were no significant changes in the body weight of the normal animals treated with vitamin C. The supplementation of vitamin E, vitamin C and *spirulina* did not restore the body weight to normal in animals exposed to 100 ppm of lead acetate. But there was a significant increase in body weight of animals exposed to 100 ppm of cadmium chloride in combination with vitamin E. This might be due to the fact that vitamin E supplementation protected the animals from the cadmium induced deleterious changes in the body weight.

A significant increase in the weight of liver and kidney was observed in animals exposed to 30 and 100 ppm of lead acetate and cadmium chloride, while the weight of lung and heart was increased only in animals exposed to 100 ppm of lead acetate and cadmium chloride. This indicated that liver and kidney are the primary organs for the toxicity of these metals rather than lung, heart and brain. This might be because of some structural changes that occurred in organs, i.e. fatty infiltration inflammation etc. The weight of liver, kidney, lung, heart and brain remained unchanged in animals after the treatment with vitamin E, vitamin C or *spirulina*. But a significant decrease in the weight of liver, kidney, lung and heart was observed in animals exposed to lead acetate and cadmium chloride in combination with vitamin C. Vitamin C is reported (Tondon, 2000) to enhance the excretion and reducing the tissue burden and reversing the lead sensitive biochemical alterations. This is because of mobilization of lead from blood, liver and kidney into urine and/or feces (Tondon, 2000). On the other hand, there were no changes in the weight of organs in animals exposed to 100 ppm of lead acetate and cadmium

chloride in combination with vitamin E or *spirulina*. An increase in weights of organs has been reported after exposure to lead acetate and cadmium chloride (Columbano et al., 1982; Choie and Richter, 1974; Boisset and Boudene, 1981).

Lipid peroxidation is a destructive free radical mediated process for biological membranes, which has been implicated in a variety of disease state. It involves the formation and propagation of lipid radicals, the uptake of oxygen, a re-arrangement of the double bond in unsaturated lipids that results in the variety of degraded products like alkenes, malondialdehyde, lipid hydroperoxide and conjugated diene and eventually destruction of membrane lipids. Biological membranes are often rich in the unsaturated fatty acids and bathed in an oxygen rich, metal containing fluid. Therefore, the membrane lipids are more susceptible to peroxidative attack. In the present study, the lipid peroxidation levels in liver, kidney, lung and heart were found to be increased significantly in animals exposed to 10, 30 and 100 ppm of lead acetate as compared to control animals. Similarly, the liver, kidney, lung and heart of animals exposed to 30 and 100 ppm of cadmium chloride were found to contain significantly higher amounts of lipid peroxidative products.

Increased lipid peroxidation can result in change in cellular metabolism of various tissues. An increase in accumulation of malondialdehyde, hydroperoxide and conjugated diene in the cells can result into cellular degradation, some biochemical and functional changes and even cell death. Accumulation of malondialdehyde, hydroperoxide and conjugated diene may be due to the movement of phosphotidyl serine (PS) and phosphotidyl ethanolamine (PE) from its inner bilayer to outer bilayer. This may be one of the possible mechanisms by which peroxidative damage and accumulation of lipid peroxidative products that are formed during the lipid peroxidation (Cheeseman and Slater, 1993). This can also cross-link and forms a base between PS and PS, PE and PE, and PS and PE. Of this chemical cross-linking between two membranes, lipid is not uniform across membrane bilayer. Physical forces building the lipid-lipid and lipid-protein may be disturbed and this may also contribute to destabilization of membrane lipid symmetry, which may be due to the accumulation of malondialdehyde in the cells. In the present study, it was observed that exposure to lead and cadmium induced lipid peroxidation in liver, kidney, lung and heart of rats. But there was no significant

change in the lipid peroxidation levels in brain indicating that both these metals might be acting on brain by some different pathways i.e. competing with calcium ions (Gelman and Michaelson, 1979; Gelman et al., 1979).

Lead and cadmium increases the generation and release of reactive oxygen species, which plays a key role in kidney damage. Lipid peroxidation by these species in the cells may cause disruption of the cellular structure integrity and capacity. These disturbances in the cell affect the cellular transport of required substances and energy production, especially in proximal tube segment. Additionally, they may induce reduction in glomerular blood flow and glomerular filtration rate through liberation of bioactive vasoconstrictor lipids (prostaglandin etc.). There is a possibility of releasing nitric oxide by stimulating nitric oxide synthase. Lead and cadmium have been reported to alter the rennin-angiotensin activity thereby affecting the renal and circulatory systems (Skoczynska, 1995).

Stimulation of lipid peroxidation by lead and cadmium in pulmonary smooth muscle microsomes appear proximal to the production of a variety of mediators that contributes to the lung injury (Ghosh et al., 1996; Schluter et al., 1995). There are reports (Manca et al., 1994) demonstrating a dose-related, non-linear evolution of total lung thiobarbituric acid reactive substances. The possible involvement of inflammatory phenomena as the most likely events responsible for the stimulation of lipid peroxidation in lung tissue following exposure to cadmium salts has also been reported. It is also well known that lung is rich in phospholipids, and the levels of phospholipid increases during peroxidic decomposition of different sub-organelles and plasma membrane lipids (Bopanna et al., 1998). The generation of peroxides may stimulate phospholipase A_2 on the cell membrane. There is a linear relationship between the stimulation of phospholipase A_2 and membrane lipid peroxidation (Selvam and Devraj, 1996; Wegner et al., 1992). Production of phospholipase A_2 causes release of a variety of eicosanoids, which are responsible for different metabolic disorders and cell injury. The increase in the levels of malondialdehyde, hydroperoxide and conjugated diene in heart suggest that lead and cadmium can produce a range of enzymatically damaging consequences such as those dependent on thiol oxidation or thiol adduct formation (Patra and Swarup, 2000). An increase in lipid peroxidation levels in heart also suggests that the

myocardial damage may be irreversible as seen in myocardial infarction (Daga et al., 1999).

It is well known that vitamin E and vitamin C are the antioxidant vitamins that protect the cells from free radical attack. In present study, vitamin E and vitamin C have shown the beneficial effect on the lead and cadmium induced lipid peroxidation. Vitamin E, vitamin C and *spirulina* decreased the elevated levels of lipid peroxidation in liver, kidney, lung and heart of the animals exposed to 100 ppm lead acetate and cadmium chloride. Vitamin E inhibited the lipid peroxidation process more effectively than vitamin C; this might be because of lipophilic nature of vitamin E and generation of tocopherols by vitamin C itself (Packer et al., 1979). Moreover, *spirulina* was also found to be more effective in decreasing the accumulation of lipid peroxides in the organs than vitamin C. This might be because of its beta-carotene content in addition to proteins and superoxide dismutase enzyme in *spirulina.* It has been reported that, vitamin E supplementation protects the animals from lead and cadmium induced lipid peroxidation (Patra et al., 2001; Krajcovicova-Kudlackova and Ozdin, 1995; Warren et al., 2000; Vaziri et al., 1999; Upasani and Balaraman, 2001b; Upasani et al., 2001). Similarly, administration of vitamin C showed a free radical scavenging activity reducing the lipid peroxidation and restoring endogenous antioxidant levels to normal in animals exposed to lead and cadmium (Tondon and Singh, 2000; Patra et al., 2001; Hudecova and Ginter, 1992; Upasani and Balaraman 2001b and Upasani et al., 2000). In addition to these vitamins, *spirulina* was also reported to have an antioxidant effect (Shastri et al., 1999; Upasani and Balaraman, 2001a; Upasani et al., 2001; Upasani and Balaraman, 2001b) in lead and cadmium induced oxidative stress.

Superoxide dismutase metabolizes superoxide anion radical. 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 superoxide dismutase levels in liver, kidney, lung and heart were decreased significantly in animals exposed to 10, 30 and 100 ppm of lead acetate and cadmium chloride. This may be attributed to the release of superoxide radical in the organs. On the other

hand, the superoxide dismutase levels were restored to normal in vitamin E, vitamin C or *spirulina* treated animals.

Catalase has been reported to be responsible for detoxification of hydrogen peroxide (Brener and Alison, 1953). Catalase may function to protect the cells against onslaught of horrendous amounts of hydrogen peroxide. Catalase deficient organisms are more rapidly killed by hydrogen peroxide (MamEation, 1990). The superoxide dismutase, catalase and reduced glutathione levels were reported to be decreased in rats exposed to lead and cadmium with an increase in lipid peroxidation (El-Missiry, 2000; Thevenod, 2000). In the present study, the catalase levels in liver, kidney, lung and heart were decreased significantly in animals exposed to lead and cadmium (10, 30 and 100 ppm). This indicates formation of higher amounts of hydrogen peroxide in the organs. Co-administration of vitamin E, vitamin C or *spirulina* restored the levels of catalase to normal in organs of rats.

Reduced glutathione is a protective molecule against chemical induced cytotoxicity (Orrenius and Moldeus, 1984). Glutathione exists in the reduced and disulphide forms. The relationship between these two forms has an important effect on the redox status of protein thiols. Thus, it has been suggested that the oxidation-reduction status of glutathione may act as a third messenger in either enhancing or diminishing the activities of a variety of biological processes, such as enzyme catalysis, protein synthesis and receptor binding (Glibert, 1982). The liver, having the greatest content of glutathione, is the major organ involved in the elimination and detoxification of xenobiotics, and it also plays a central role in the inter-organ relationship of glutathione by its ability to export glutathione into plasma at a rate that accounts for nearly all of its hepatic biosynthesis (Kaplowitz et al., 1985). Reduced glutathione metabolism plays a vital role in may biological processes, in detoxification of xenobiotics, hydrogen peroxide and various reactions of oxygen free radicals (Meister, 1984). It has been reported that the GSH levels decreases rapidly in animals exposed to lead (Gurer et al., 1998) and cadmium (Karmarkar et al., 1998). Similar results were observed in this present study. The levels of reduced glutathione in liver, kidney, lung and heart were decreased significantly after thirty days exposure to lead and cadmium (10, 30 and 100 ppm). Conversely, simultaneous administration of vitamin E, vitamin C or *spirulina* have restored the

levels to normal in the animals exposed to 100 ppm of lead acetate and cadmium chloride. This indicates the protective antioxidant effect of vitamin E, vitamin C or *spirulina*. The modulatory effect of vitamin E and vitamin C on lead and cadmium toxicity may be attributed to lipid peroxidation chain breaking reactions (Packer, 1979), while the protective effect of *spirulina* is probably due to presence of high proteins, beta-carotene and superoxide dismutase enzyme (Shastri et al., 1999).

Glucose-6-phosphate, an important gluconeogenic enzyme, occurs almost exclusively in the microsomes of the liver, kidney and small intestine, where it catalyses the hydrolysis of glucose-6-phosphate. In the present study, the glucose-6-phosphate dehydrogenase levels in liver were decreased significantly in the animals exposed to lead (10, 30 and 100 ppm) and in cadmium (30, 100 ppm). This decrease may be attributed to the feedback by higher levels of lipids in the liver. Lead and cadmium exposure might be affecting glycolytic and other oxidative pathways. In this present study, the glucose-6-phosphate dehyrogenase levels in liver were increased significantly due to administration of vitamin E, vitamin C and *spirulina* to animals exposed to 100 ppm of lead acetate. Vitamin E and *spirulina* significantly restored the levels of glucose-6-phosphate dehyrogenase to normal in animals exposed to cadmium. The increase in the glucose-6-phosphate dehyrogenase level is reported to stimulate the pentose monophosphate shunt pathways and thereby causes elevation in the glutathione and its related enzyme levels (Chitra and Leelamma, 1999). It is reported (Chow et al., 1974) that increase in glucose-6-phosphate dehyrogenase levels elevates glutathione levels, which plays an important role for protecting the cells from lipid peroxide induced damage through the formation of reducing compounds such as NADPH. (Kaplowitz et al., 1985).

The lipid dependent membrane bound –SH group containing enzymes are ATPases; once activated, they play a very important role in the active transport of cations (Luly et al., 1972). Any alteration in membrane lipids leads to change in the membrane fluidity, which in turn alters the ATPases activity and cellular function. 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 (Schuurmans-Steknoven and Bonting, 1981). Membrane cholesterol and

phospholipids are reported to decrease the enzyme activity (Kumari et al., 1990). The biological membranes like erythrocytes exhibit two Ca^{++}-ATPases components, which differ with respect to their affinities for calcium ions. One of them is low-affinity Ca^{++}-ATPase, which is responsible for the shape and deformability of the erythrocyte membrane. The second type of Ca^{++}-ATPase is called high-affinity Ca^{++}-ATPase, which is responsible for the active outward transport of the calcium ions across the biological membranes to maintain the very steep calcium ion concentration gradient between the inside and outside of the biological membrane. These require higher amounts of calcium (10 mM) for their activity (Hjerten and Pan, 1983). Any imbalance in the calcium ions leads to the change in the activity of Ca^{++}-ATPase. Lead and cadmium are reported to interfere with the activity of calcium in the body, thereby affecting the levels of these ATPases. The presence of Mg^{++}-ATPase has been reported in the plasma membrane, liver, kidney etc. Mg^{++}-ATPase is often referred to as a basic ATPase (Ohinishi et al., 1982). In lead poisoning, there is a marked decrease in the ATPases activity of liver and kidney (Jarrar and Mahmoud, 2000; Upasani and Balaraman, 2001b). In the present study, there was a significant decrease in the Na^{+}-K^{+}-ATPase, Ca^{++}-ATPase and Mg^{++}-ATPase levels in liver and kidney of animals exposed to lead (10, 30 and 100 ppm). On the other hand, the Na^{+}-K^{+}-ATPase, Ca^{++}-ATPase and Mg^{++}-ATPase levels in liver and kidney were decreased in animals after thirty days exposure to cadmium (30 and 100 ppm). In addition there was also a significant decrease in Ca^{++}-ATPase levels in kidney in animals exposed to 10 ppm of cadmium chloride. This indicated that cadmium at lower concentration (10 ppm) did not alter the ATPases levels in liver and kidney. This might be due to the utilization of significant amounts of cadmium either for the formation of metallothionein or due to lesser quantity reaching the system. On the other hand, lead at 10 ppm concentration has shown significant decrease in all ATPases in liver and kidney. The heavy metals like lead, cadmium and mercury show toxicity by interfering or causing deficiencies of nutritionally essential metals (Goyer, 1973). Lead competes with calcium, inhibiting the release of neurotransmitters and interferes with the regulation of cell metabolism. This is because of binding of lead to second messenger calcium receptors or by blocking calcium transport by decreasing the efficiency of calcium channel and Ca^{++}-ATP pumps. This leads to increase in uptake of lead by the calcium binding sites and

uptake by mitochondria (Goyer, 1973). Similarly, cadmium competes with zinc for binding sites on metallothionein.

ATPases are more likely to be disturbed with the change in the levels of lipids. The decrease in levels of ATPases may be due to the changes in the levels of cholesterol, phospholipids and triglycerides in animals exposed to lead and cadmium. These changes in the levels of lipids and ATPases are expected in the oxidative stress (Pal et al., 1993). In this present study the results are in agreement of previous studies indicating the decrease in the ATPases levels were due to lead and cadmium.

Vitamin E, vitamin C or *spirulina* restored the Na^{+}-K^{+}-ATPase, Ca^{++}-ATPase and Mg^{++}-ATPase levels in liver and kidney in animals exposed to lead, while vitamin E and *spirulina* restored the levels of Na^{+}-K^{+}-ATPase only in liver and kidney of animals exposed to cadmium. Further, it was noticed that the Ca^{++}-ATPase levels in liver and kidney were restored to normal in animals exposed to cadmium by vitamin E, while vitamin C or *spirulina* have been effective in elevating the decreased Ca^{++}-ATPase levels in kidney only. On the other hand, vitamin E treatment restored the Mg^{++}-ATPase levels in liver and kidney in animals exposed to cadmium, while *spirulina* treatment restored the Mg^{++}-ATPase levels in kidney to normal. But vitamin C was not effective in protecting the levels of these ATPases in liver and kidney of animals exposed to cadmium.

The membrane bound ATPases is attributed to the presence of lipids especially phospholipids. In addition to the lipids, the ATPases activity also depends on various cations i.e. sodium, potassium, calcium and magnesium along with activation of –SH group. Lead and cadmium are known to alter the lipid levels, thereby affecting the membrane integrity and fluidity (Upasani et al., 2001b). It is evidenced in this present study that, supplementation of vitamin E, vitamin C or *spirulina* to the animals exposed to lead and cadmium corrected the disturbed lipid levels. Vitamin E and vitamin C, which shows free radical scavenging activity, regularizes the lipid levels there by preventing the inactivation of –SH group and stabilizing the membrane bound enzymes (Tanaka and Strickland, 1965). *Spirulina* is rich in proteins, minerals and vitamins (nicotinic acid, vitamin C etc.), which

show antioxidant property. This might be the reason for protection of membrane bound enzymes by vitamin E, vitamin C and *spirulina*, which restored the levels of membrane bound enzymes to normal in animals exposed to lead and cadmium.

Proteins are the essential building blocks of the cell or organ. Proteins play an important role in the functioning of organs. In the present study, the protein levels in liver, kidney, lung and heart were decreased significantly in animals exposed to 30 and 100 ppm of lead acetate and cadmium chloride. It was further observed that, vitamin E, vitamin C or *spirulina* treatment restored the protein levels to normal in liver, kidney, lung and heart of animals exposed to lead acetate and cadmium chloride. Protein levels are found to change in majority of conditions like shock, chronic diseases etc. This might be because of two phenomena's; the first is catabolism of proteins to amino acids by oxidation of various cell organelles and second phenomenon may be the formation of stress protein, a protective mechanism. Dhar and Banerjee (1979) have reported that, the metals (lead) are responsible for diminution of the protein content of the liver and kidney. The changes in the concentration or levels of proteins may cause disturbances in other cellular components such as DNA, RNA etc. along with some enzymes. In the present study, the protein levels were decreased significantly by lead and cadmium in various organs of animals and supplementation of antioxidants resulted into normalization of the decreased protein levels in lead and cadmium intoxicated animals.

Lipids are the important constituents of the organs. Lead and cadmium cause pro-oxidant and peroxidative damage to membrane fragility and permeability is a likely consequence of lead and cadmium poisoning. The present study demonstrated a significant increase in the cholesterol, triglyceride and phospholipid levels in liver, kidney, lung and heart in animals exposed to lead (10. 30 and 100 ppm) and cadmium (30 and 100 ppm). The cholesterol levels in kidney were significantly increased in animals exposed to 10 ppm of cadmium. This may be attributed to the ability of lead and cadmium to alter the cellular structures. Other reason for increase in the levels of lipids may be increase in the peroxidative processes by release of free radicals. The lipid peroxidation process is a chain reaction which progresses by entry of free radical into the lipid molecules and abstracts a

hydrogen atom from a fatty acid - allylic methylene (-CH_2-) group. This produces a carbon centered radical that stabilizes by rearrangement to form a conjugated diene. This process is favored in carbon hydrogen bonds adjacent to unsaturated double bond and is also stimulated by hydroxyl radicals. The metal ions play a role of template for the formation of hydroxyl radicals (Maxwell and Lip, 1997). It is reported that lead and cadmium exposure causes dose dependent increase in the cholesterol, triglyceride and phospholipid levels in the liver and other organs (Honchal et al., 1991; Lawton and Donaldson, 1991; Skoczynska and Smolik., 1994). The increase in cholesterol, triglyceride and phospholipid indicates lipid abnormalities in the organs. It is known that triglycerides rapidly metabolize in blood to form free fatty acids, which are vulnerable to redox reactions. Cholesterol is a sterol with an ability to affect the molecular motion of hydrocarbon chains of lipids in bilayers of cell membranes. Overall oxidative modification of lipid starts during the oxidative stress. On the other hand, vitamin E, vitamin C and other antioxidants protects the process of oxidative modification of lipids (Retsky et al., 1993). The vitamin E scavenges these free radicals, thereby preventing the oxidative modification of lipids. Vitamin E is a lipid soluble vitamin which plays an important role in breaking the chain reaction of lipid peroxidation. The conversion of vitamin C into vitamin E and co-enzyme Q10 are associated with the vitamin E mediated free radical quenching. Vitamin C is a strong electron donor, rapidly oxidizes leading to transient formation of dehydro-L-ascorbic acid, and utilizes the oxygen formed in the organ; thereby it protects the oxidative modification of lipids. A natural compound like *spirulina* is also reported to reduce the oxidative modification of lipids and protect the cells from free radical induced oxidative stress. In addition to this, *spirulina*, a protein rich algae, also contains high amounts of vitamins, especially, nicotinic acid. The lipid lowering effect of nicotinic acid is well reported in the literature (Demaagd, 1999). In the present study, vitamin E, vitamin C and *spirulina* have been shown to normalize the cholesterol, triglyceride and phospholipid levels in liver, kidney, lung and heart in animals exposed to lead. Similarly, vitamin E or *spirulina* administration has significantly decreased the elevated lipid levels in organs of animals exposed to cadmium, indicating the protection against the oxidative modification of lipids.

Transaminases are involved in the intermolecular transfer of an amino group from a donar α- amino acid to an acceptor α- keto acid without formation of ammonia. Transamination plays a key role in intermediary metabolism as it provides a means for the synthesis and degradation of amino acids in living cells. The aspartate transaminase or glutamate pyruvate transaminase (GPT) activity is widely distributed in serum and in tissues. Fluctuations in their levels indicate major metabolic problems in the body. In the present investigation there was a significant increase in GPT levels in serum of animals exposed to lead acetate (10, 30 and 100 ppm) and cadmium (30 and 100 ppm). Similarly, the glutamate oxaloacetate transaminase (GOT) levels in serum were significantly increased in animals exposed to lead acetate (30 and 100 ppm) and cadmium chloride (100 ppm). Vitamin E or *spirulina* treatment restored the elevated GPT and GOT levels in serum of animals exposed to lead and cadmium. The lead and cadmium induced elevation of GPT and GOT have been well reported (Othman and El-Missiry, 1998; Shimizu and Morita, 1990). Earlier reports have demonstrated the increase in GPT and GOT levels in serum after single does of lead and even after chronic low-level exposure to lead and cadmium (Columbano et al., 1983; Dhar et al., 1980; Lupo et al., 1986). The increase in these liver enzymes is attributed to injury and synthesis from hepatocytes. The leakage of these enzymes from damaged membrane is very high (as in case of damaged organ) and appears in serum rapidly (Pappas, 1986). The present study has shown that there is damage to the membranes of various organs, which have eventually elevated the serum levels of GPT and GOT enzymes. A number of antioxidant and indigenous compounds have been reported to reduce the GPT and GOT levels by correcting the membrane damage. In the present study, vitamin E, vitamin C protected the organs from injury because of their antioxidant properties. *Spirulina* is also found to correct the cellular abnormality and maintained the GPT and GOT levels to normal in rats exposed to carbontetrachloride and d-galactosamine (Murugan, 1995).

Phosphatases are a group of relatively non-specific enzymes, which hydrolyze a variety of ester orthophosphates under alkaline (Alkaline phosphatase) or acidic (Acid phosphatase) conditions. Acid phosphatase is a lysosomal enzyme while alkaline phosphatase is a membrane bound enzyme. Increase in alkaline

phosphatase occurs mainly in bone disease and in diseases of liver and biliary tract. Changes in alkaline phosphatase values results mainly due to reduction of –SH groups. Most of the increase may follow as a result of cholestasis induced by any toxicant including lead and cadmium as a consequence of hepatocellular toxicity. There was a significant increase in alkaline phosphatase and acid phosphatase levels in serum of animals exposed to lead (10, 30 and 100 ppm) and cadmium (30 and 100 ppm). Therefore it can be stated that, liver is at high risk of peroxidative attack due to the lead and cadmium exposure. The elevated levels of alkaline phosphatase and acid phosphatase were reported earlier in lead and cadmium exposure. This indicates some degenerative changes in the organs especially in liver (Othman and El-Missiry, 1998). Jarrar and Mahmoud (2000) have reported elevated alkaline phosphatase and acid phosphatase levels in animals intoxicated with lead. Vitamin E, vitamin C or *spirulina* down regulated the elevated levels of alkaline phosphatase and acid phosphatase in animals exposed to lead and cadmium. These antioxidants ameliorate the augmented levels of alkaline phosphatase and acid phosphatase by restoring the levels of membrane bound enzymes, lipids and correcting the cytosolic disturbances in lead and cadmium exposed rats.

All these elevated levels of transaminase and phosphatases suggests that normal metabolizing capacity is diminished in liver and other organs by lead and cadmium intoxication.

Lactate dehydrogenase is another cytosolic enzyme, which catalyzes the irreversible oxidation of L-lactate to pyruvate. Lactate dehydrogenase determination is of clinical significance in the diagnosis of various diseases. Elevated levels of lactate dehydrogenase are indicative of myocardial infarction. Elevation of serum lactate dehydrogenase is also observed in liver diseases and certain types of carcinomas. Lactate dehydrogenase catalyses the oxidation of L-lactate to pyruvate in the presence of the coenzyme NAD. The resulting NADH is then oxidized in the presence of an electron-transfer agent. Similar mechanism is occurring rapidly in presence of free radicals that are electron transfer agents. Release of lactate dehydrogenase from the cell has been increased in animals exposed to lead (100 ppm) and cadmium (30 and 100 ppm). Treatment with

vitamin E, vitamin C or *spirulina* to the animals exposed to lead and cadmium have restored the levels of lactate dehydrogenase to normal in serum, indicating interference in the electron transfer stage of NADH oxidation, thereby protecting organs from oxidative damage induced by lead and cadmium. Elevated levels of lactate dehydrogenase have been reported earlier (Colderon-Salinas et al., 1993; Sakaguchi et al., 1982) in lead and cadmium toxicity.

The bilirubin levels in serum were increased significantly in animals exposed to 30 and 100 ppm of lead acetate and cadmium chloride, while treatment with vitamin E, vitamin C or *spirulina* have significantly decreased the elevated levels of bilirubin in serum of animals exposed to lead and cadmium. Similarly, protein levels of serum were decreased significantly in animals exposed to lead and cadmium (30 and 100 ppm). Treatment with vitamin E, vitamin C and *spirulina* were found to restore the protein levels to normal in serum of animals exposed to lead, while vitamin E and *spirulina* were found to be effective in correcting the decreased protein levels to normal in animals exposed to cadmium. It is well known that vitamins are the molecules that stimulate the process of anabolism. *Spirulina* being a protein rich natural material is reported to elevate the protein levels in animals and in humans as well (Jassby, 1988; Annapurna et al., 1991a, 1991b).

The cholesterol levels in serum were decreased significantly in animals exposed to 30 and 100 ppm of lead and cadmium. But treatments with vitamin E, vitamin C or *spirulina* have corrected the cholesterol levels to normal in rats intoxicated with lead and cadmium. The triglyceride levels in serum were increased significantly in animals exposed to lead and cadmium (30 and 100 ppm), while the phospholipid levels were increased significantly in animals exposed to lead and cadmium (10, 30 and 100 ppm). Treatments with vitamin E, vitamin C or spirulina have restored the levels of triglycerides and phospholipids to normal in lead and cadmium-intoxicated animals. Earlier reports suggest that exposure to lead and cadmium decreases the cholesterol levels (Skoczynska et al., 1993) while the lipogenic action of lead and cadmium suggests the increase in the levels of triglycerides and phospholipids in serum (Honchel et al., 1991; Bresslar et al., 1994). It has been also reported that, increase in triglyceride and phospholipid levels alters the

permeability of the capillaries. A possible mechanism for this alteration may be related to the ability of lead and cadmium to substitute the calcium. Products derived from phospholipid metabolism are eicosonoids and diacylglycerol that control endothelial permeability. These are partly regulated by the intracellular calcium ions and thus may be sensitive to lead and cadmium. The present study is in agreement with earlier findings. Triglyceride once separated from the membranes gets metabolized to free fatty acids which generally undergo oxidative modifications of the lipids.

Chronic consumption of lead and cadmium via drinking water resulted in a dose dependent accumulation of these metals in vital organs. There was a dose dependent significant accumulation of lead and cadmium in liver, kidney, lung, heart and brain. This is because of high levels of metals available in the body for a long time. The presence of these metals in the body is a current topic of concern. This is attributed to their long biological half life. Metals are known to have a longer biological half life thereby they remain a potential threat to the vital organs for a long time (Friberg et al., 1986). It is reported that the deficiency of some dietary constituents plays an important role in the absorption of these metals. It has been long known that dietary calcium deficiency enhances lead and cadmium absorption (Six and Goyer, 1970b). It is also known that the deficiency of dietary phosphorus plays a vital role in absorption of lead at a greater efficiency (Barton and Conrad, 1981). The vulnerable site for localization of lead and cadmium is the calcified matrix of the skeleton. But the major concern has been with the disposition of lead and cadmium in the other more toxicologically significant tissues, e.g. liver, lung, heart, kidney, brain and hemopoietic system.

It has been also reported that, diet rich in some chelating agents, complexes, calcium etc. decreases the absorption of these metals. But in the present study, it was observed that; vitamin C has significantly decreased the accumulation of these metals in liver, kidney, lung, heart and brain of animals exposed to lead and cadmium. Chemically, vitamin C is ascorbic acid, an antioxidant, and the probable reason for decreasing the accumulation of these metals in organ may be its complex formation with the metals thereby decreasing the accumulation of lead in the body (Dalley et al., 1989). However, vitamin E or *spirulina* supplementation

did not alter the accumulation of these metals in vital organs like liver, kidney, lung, heart and brain. It was interesting to note that supplementation with *spirulina* significantly decreased the deposition of lead in brain of animals exposed to lead acetate suggesting *spirulina* protected the brain from lead accumulation. The exact action and mechanism by which this protection is taking place will be the new area of research.

The high degree of accumulation of these metals may be the probable reason for the pathological changes that have occurred in the liver, kidney, lung and heart of animals exposed to lead and cadmium. Histopathological examination of liver, kidney and heart showed marked pathological changes after chronic low-level exposure to lead and cadmium. These changes were found to be dose dependent.

Significant changes were observed in liver of animals exposed to 100 ppm of lead acetate and cadmium chloride. The fatty infiltration and hydropic changes were observed in the hepatic cells. The deposition in lipids of the cells has significantly contributed to liver weight gain. The increase of weight in liver is also due to inflammation and proliferation of hepatocytes. The presence of WBCs indicates on going active destruction of the liver cells. The inflammatory response in liver and its repair (liver cell regeneration) following exposure to lead and cadmium have been shown to mediated by cytokines (Dong et al., 1998; Ledda-columbano et al., 1983; Kayama et al., 1995). The acute inflammatory cytokines formation process is reported to be mediated by oxidative stress (Dong et al., 1998; Lawton and Donaldson, 1991). Necrotic changes in liver are associated with changes in the liver dehydrogenease enzyme. It is observed in the present biochemical estimations that G-6-PD enzyme is depressed in lead and cadmium exposed rats. The liver necrosis is normally of two patterns; fragmentation and condensation. The cytoplasm may become intensely eosinophilic and the nuclear fragments dark and shrunken. The fragmented portions may get phagocytocized or extruded into the adjacent spaces of bililary canaliculus. Lipid accumulation in the inter-cellular spaces is because of leakage of lipids from the membranes of the parenchymal cells due to high permeability and diminished activities of membrane bound enzymes as evidenced in the present study. It is also found that the fat spaces represented many cells. There is evidence of bi and trinucleate cells. They may be

due to recovery phase and may provide evidence of regeneration. The result of the present study demonstrated that there is a liver injury due to the exposure to lead and cadmium. It was also supported by the biochemical investigations like increased levels of transaminases, phosphatases and lactate dehydrogenase in serum of lead and cadmium exposed rats along with elevation of some marker enzymes and products of oxidative stress. Further, it also demonstrates that, the injury is mediated through the release of toxic oxygen free radials. It has been reported that parenchymal cell necrosis with marked elevation of serum enzymes is the major feature of lead and cadmium toxicity (Columbano et al., 1983; Goyer and Rhine, 1978; Dudly et al., 1984; Habeebu et al., 2000) which indicates the oxidative stress and damage to the biological membranes. In the present study, supplementation of vitamin E, vitamin C or *spirulina* have shown beneficial effects on the liver by protecting the hepatic cells from injury. The protection by vitamin E, vitamin C or *spirulina* was also evidenced by reduction in the numbers of pyknotic nuclei in the hepatic cells and a decrease in the fatty infiltration, hydropic changes and inflammation in the hepatic cells. The hepatomegaly was not prominent in vitamin E, vitamin C and *spirulina* treated animals as compared to lead and cadmium exposed animals. This might be because of protective antioxidant action of vitamin E, vitamin C or *spirulina*. It is reported that vitamin E is a potent inhibitor of oxidative stress, vitamin C forms an active metabolite that protects the liver from injury and *spirulina* a protein rich diet, having higher amounts of beta-carotene and superoxide dismutase enzyme thereby protecting the liver.

The histopathological examination of kidney showed enlargement of kidney in lead and cadmium exposed animals. This might be because of fat deposition. The kidney tubules were bigger in size as compared to control kidney tubule; this enlargement of the tubules along with some leukocytes was indicated the swelling and inflammation. Reports confirming the histopathological effects of oral lead and cadmium in various organs of animals are well addressed. This might be because of their long biological half-life and elevated tissue burden (Friberg et al., 1986) and kidney is the major site of lead and cadmium accumulation (Goyer and Cherian, 1995). In the present study, there are prominent histopathological changes in renal cells, which might be attributed to increased renal tissue burden by lead

and cadmium. There is also evidence that lead and cadmium may be associated with renal neoplasia in humans and rodents (Goyer and Cherian, 1995). It is well known that the toxic effects of lead and cadmium are dependent on the dose, duration and route of exposure. Enlargement of kidney is indicative of inflammatory and proliferative lesions following repeated oral exposure to lead and cadmium. The renal injury caused by lead and cadmium exposure in animals could have potentially resulted due to the increased oxidative stress by these metals (Liu et al., 2000). It has been proposed that oxidative damage is involved in the chronic lead and cadmium induced nephropathy (Bagchi et al., 1997; Shaikh et al., 1999). In the present study, administration of vitamin E, vitamin C or *spirulina* has significantly reduced the kidney damage in animals exposed to lead and cadmium. Supplementation of vitamin E and vitamin C increases the resistance to oxidative stress (Liu et al., 2000), thereby protecting the renal cells from the damage by such pro-oxidants. This is also evidenced by decrease in the weight of kidney in animals exposed to lead and cadmium in combination with vitamin E, vitamin C or *spirulina* as compare to kidney of animals exposed to lead and cadmium alone. The supplementation of vitamin E, vitamin C or spirulina has protected the kidney from damage by lead and cadmium in terms of decrease in swelling and fat deposition.

In the present study, there was hyperemia along with the interstitial inflammation in lungs of animals exposed to lead acetate and cadmium chloride, indicating the pulmonary damage due to exposure to lead and cadmium. These changes were pronounced in animals exposed to cadmium than lead. This might be because of higher deposition of lead and cadmium in the pulmonary region. There was a generalized widening of the alveolar septa, indicated by the enlarged alveolar area. The peribronchial lymphoid hyperplacia was observed in the lung of animals exposed to lead and cadmium, which might be because of lymphocytic infiltration in the tissues. Supplementation of vitamin E and vitamin C completely protected the lung from the aforementioned toxic effects due to the exposure of lead and cadmium. But, supplementation of *spirulina* could not prevent the hyperemia and lymphocytic infiltration in the lungs.

There was inflammatory invasion of WBCs and shrinking of the cardiac muscle was observed. There was a significant enlargement of heart. The mild degree of cellular swelling, which is known as the minor response to injury and cytoplasmic disruption were observed in the cardiac cells of animals exposed to lead and cadmium. The disruption of the cytoplasm was observed which might be because of the action of these toxic metals. Supplementation of vitamin E and vitamin C have significantly reduced the lead and cadmium induced histopathological changes in heart in terms of decreased swelling and cytoplasmic disruption. Supplementation of *spirulina* to the animals exposed to lead and cadmium has significantly decreased the cytoplasmic disruption in the heart but no change in the cellular swelling.

Though brain is the target organ for lead induced neurological changes, the present study did not show any alteration of the enzymes and cellular constituents in these organs. The lead induced neurological changes might be due to different mechanism rather than the oxidative stress.

The biochemical, pharmacological and toxicological study on lead and cadmium showed that these metals caused oxidative stress and administration of vitamin E, vitamin C and *spirulina* largely reduced this oxidative stress by virtue of their antioxidant mechanism.