CHAPTER - 5

DISCUSSION

There was a significant decrease in the body weight of animals after 30 days of exposure to cadmium chloride in the strength of 100ppm compared to the control. However, no significant changes were observed in the body weight of animals after thirty days of exposure to 10 and 30 ppm of cadmium chloride. The probable reason for the same are the dose related metabolic effects of cadmium. These results are in line with previous studies (Institoris et al. 1993 and Patra et al. 2001). Metabolic effects following ingestion of heavy metals have been found to be dose dependant (Columbano et al. 1983; Choie and Richter, 1974 and Goyer, 1995) in their respective studies. The supplementation of selenium, alpha lipoic acid and coenzyme Q10 to cadmium significantly helped to prevent the weight loss in animals. Selenium probably with its ability to form inert complex with cadmium decreased the load of the toxic drug both in the body and different tissues. Coenzyme Q10 by being universally distributed in the body and its effect on oxidative phosphorylation in the mitochondria helps in regularizing the distorted metabolic processes by cadmium and is beneficial in preventing weight loss.Dihydrolipoic acid (DHLA) reduced form of ALA is capable to regenerate ascorbic acid which is reported to reduce the tissue burden of heavy metals (Tondon and Singh, 2000).

A significant increase in the weight of liver, kidney, heart, and lung was observed in animals exposed to 100ppm of cadmium chloride. This might be due to fatty infiltration, hydropic changes leading to retention of fluids and infiltration by inflammatory cells in form of neutrophils and lymphocytes. There was also proliferation of basic cells eg. hepatocytes in the liver as a part of the reparative mechanism following damage by cadmium leading to an increase in the mass and weight of the organs. An increase in the weight of organs following administration of heavy metals has also been observed by others (Columbano et al., 1983; Choie and Richter, 1974; Boisset and Boudene, 1981)

The heart rate of the animals exposed to cadmium chloride (100 ppm) for 30 days was significantly increased. Similar results were also observed in previous studies (Puri, 1997). However there were also contradictory findings. Ozturk et al. (2009) in their study concluded that though there was an increase in stroke volume and cardiac output, there was no significant change in the heart rate of animals administered cadmium. On supplementation of alpha lipoic acid, selenium and coenzyme Q10 the heart rate significantly reduced to near

normal levels in the animals exposed to cadmium chloride (100 ppm) for 30 days. This can be attributed to their antioxidant and cardioprotective effects as mentioned in the discussion below.

There are lots of discrepancies in the literature as far as the hypertensive effects of cadmium chloride are concerned.

The results of several studies suggest that persons with hypertension have more cadmium and higher cadmium to zinc ratio in their kidneys than those without hypertension. In contrast to these findings, no relationship between cardiovascular disease and cadmium levels in the kidney was found in a study of 80 individuals at postmortem (Morgan, 1969). In addition, no relationship between hypertension and urinary cadmium excretion has been observed. (Szadkowski et al., 1969)

Oral administration of cadmium has produced hypertension in animals; the dose-response curve, however, is not monotonic. The greatest effects are observed with oral doses 0.01mg/day or intra-peritoneal injections of 0.0001 to 0.001mg/kg. Doses of an order of Magnitudes higher have little effect (Kopp et al., 1982)

The induction of hypertension in animals by feeding cadmium was first reported by Schroeder and Vinton (1962). Certainly parentral administration of cadmium could induce acute transient hypertension in animals (Schroeder et al., 1966; Perry and Erlanger, 1971b). When the animals were treated with a drug that substituted zinc for the cadmium already in their tissues, blood pressures returned to normal. During the next years, Schroeder extended his initial observations in numerous reports (Schroeder, 1964; Schroeder and Buckman, 1967; Schroeder et al., 1968a; 1968b; 1970; Kanisawa and Schroeder, 1969a; 1969b). Perry and Erlanger (1971a) confirmed that chronically fed cadmium could raise systolic pressure. Schroeder et al., have reported very marked hypertension whereas Perry et al., could induce a mild elevation in blood pressure in experimental animals.

Schroeder et al. (1966) gave first report of chronic hypertension after the injection of cadmium. Cadmium acetate (2mg/kg, i.p.) was administered to female rats of the Long-Evans strain. Three weeks after a single injection of cadmium 31 percent of the rats exhibited elevation of blood pressure. When a second dose of cadmium 1mg/kg was given to the

normotensives, all exhibited hypertension after one week. When cadmium was given intraperitoneally (2mg/kg) to another group of rats with partial constriction of left renal artery, 71 percent of the rats showed severe hypertension. Cadmium has been reported to induce hypertension accompanied by decreased vascular responsiveness to autonomic drugs in the rat and the rabbit. Cadmium acetate administration to female Sprague-Dawley rats failed to elevate blood pressure, although cadmium-treated animals exhibited decreased blood pressure responses to intravenously administered norepinephrine, acetylcholine, isopoterenol and atropine (Michael Porter, Tom Miya and William Bousquet Dept of Pharmacology and Toxicology, School of Pharmacy and Pharmacological Sciences, Purdue University 1975)

However, workers like Hammer et al. (1972) found no evidence of hypertension in workers exposed to cadmium containing superphosphate dust.

Such discrepancies in the blood pressure response to cadmium chloride may be due to difference in the dosage, duration of treatment, route of administration and also species.

The authors who found development of hypertension in cadmium fed animals were unable to explain the exact pathogenesis except to say that it differs from that of renal ischemic hypertension but is associated with similar and less severe renal vascular lesions. The vascular lesions found were generalized thickening of renal arterioles and narrowing of lumen. Perry and Erlanger (1973) observed that i.p. injection of 1.8 μmoles of cadmium produced significant elevation of renin at all time intervals. These authors postulated a probable role of renin in cadmium induced hypertension. Doyle et al. (1975) observed Na^+ retention following cadmium feeding. Perry and Erlanger (1980, 1981) showed that hypertension associated with cadmium exposure could result from its anti natriuretic effect. It is not clear whether the antinatriuretic effect of cadmium is a direct effect on the kidney or due to activation of renin angiotensin system.

Balaraman et al. (1989) showed that cadmium induced pressor response as well as hypertension is prevented by calcium channel blockers like verapamil and nifedipine. It was further suggested that Cadmium might be mimicking Calcium ion as a partial agonist or altering the Calcium transport across the cell membrane.

Atrial natriuretic peptide (ANP), a hormone secreted by the heart, has been established as a diuretic, natriuretic, smooth muscle relaxant and may be a modulator of central cardiovascular regulation. Cadmium can alter select tissue content of the ANP and this interaction may play an important role in the cardiovascular effects of cadmium (Girdhar and Isom, 1991).In addition, arterial hypertension seen during cadmium toxicity might be related to increased synthesis of epinephrine in adrenal glands (Rastogi and Singhal, 1975).

The present study also showed a significant increase in the systolic and diastolic blood pressure of rats exposed to cadmium chloride 100 ppm measured both by the cuff method. When these animals were supplemented with alpha lipoic acid, selenium and coenzyme Q10 their blood pressure significantly returned to near normal levels.

Alpha lipoic acid(ALA) may be acting in cadmium produced hypertension by having a protective effect on the kidney.One animal study suggested that ALA may be effective in the prevention of early diabetic glomerular injury and may provide more protection than high doses of vitamin C or vitamin E.(Melhem et al., 2001). The study observed ALA (30mg/kg body weight daily for two months) given to diabetic rats either prevented or significantly attenuated increases in urinary albumin excretion, fractional albumin clearance, glomerular volume, and glomerular content of immunoreactive transforming growth factor-[beta] and collagen [alpha] addition, it was found that ALA, but not vitamins C or E, significantly increased renal-cortical glutathione content. In spontaneously hypertensive rats (SHRs), excess endogenous aldehydes bind sulfhydryl groups of membrane proteins, altering membrane Ca2+ channels and increasing cytosolic free calcium and blood pressure. The thiol compound, N-acetyl cysteine, normalizes elevated blood pressure in SHRs by binding excess endogenous aldehydes and normalizing membrane Ca2+ channels and cytosolic free calcium. The study conducted by Vasdev et al. (2000) showed that dietary supplementation of an endogenous fatty acid, alpha-lipoic acid, another thiol compound that is known to increase tissue cysteine and glutathione, can lower blood pressure and normalize associated biochemical and histopathological changes in SHRs

Coenzyme Q10 appears to modulate blood pressure by reducing resistance to blood flow. Several trials have reported that supplementation with CoQ10 significantly reduced blood pressure in people with hypertension, usually after ten weeks to four or more months of treatment (Gaby, 1996). CoQ10 has been utilized to treat hypertension. The department of medicine, Mt. Sinai Hospital and Medical Center in New York, reported in the Journal of Clinical Pharmacology that its cardiovascular importance is now being realized in clinical trials worldwide (Greenberg and Frishman, 1990). In humans, a deficiency of CoQ10 was found in 39% of pátients with hypertension, compared to 6% to those with normal blood pressure. Providing these patients with 60 mg of CoQ10 for eight weeks resulted in a 10% or greater decrease in blood pressure (Yamagami et al., 1975). In a double blind study, 20 hypertensive subjects with low serum CoQ10 levels receiving 100 mg of CoQ10 per day for 12 weeks, showed a significant reduction in systolic and diastolic blood pressure (Yamagami et al., 1986). In a 1994 study, 109 patients with known hypertension were given 225 mg of CoQ10 daily, achieving a serum level of at least 2mcg/ml. There was a decrease in systolic blood pressure from an average of 159mm Hg to 147mm Hg, while mean diastolic pressures dropped from 94 to 85 mm Hg. Fifty percent of patients were able to decrease or eliminate their medication (Langsjoen et al., 1994). The mechanism by which CoQ10 reduces blood pressure is not fully understood. However in 1990, Digiesi and Cantini demonstrated a decrease in the resistance of blood vessels (Digiesi and Cantini, 1990) Further, clinical cardiologist Stephen Sinatra, MD, FACC, believes this action may be secondary to an improvement in the metabolic function of the cells, and that the antioxidant properties of CoQ10 may help normalize cellular chemistry and promote optimal tone and compliance of the elastic vessel walls (Sinatra and Steven, 1998).

The theory that selenium and cadmium can form complexes has been substantiated by researchers in animal studies with concomitant selenium and cadmium exposure. In a study with acute cadmium toxicity (8mg/kg oral cadmium) and concomitant oral selenium supplementation (350μg/kg sodium selenite), rats who received both had a 25-percent reduction in kidney cadmium.In addition selenium treatment was postulated to protect the kidney tissues against the toxicity of cadmium since it reduced MDA levels and increased the activities of antioxidant enzymes in these tissues. (El-Sharaky et al., 2007). Thus by

decreasing the tissue load of cadmium in tissues particularly the kidney, selenium must be having a role in reducing the increased blood pressure to normal in cadmium treated animals.

In our study there was increased vascular reactivity to adrenalin, noradrenalin and isoprenalin in animals fed with cadmium chloride. There are many studies in the literature which have had the same findings and they have also postulated the mechanism by which there is an increase in the pressor response by cadmium. Perry and Erlanger (1975) suggested a direct vasoconstriction as a mechanism of cadmium induced pressor effect. Nechay et al. (1978) have observed that rats treated chronically with low dose of cadmium showed potentiation of the pressor response to noradrenalin. Fadloun and Leach (1981) suggested the involvement of the sympathetic system and Caprino et al. (1982) showed the role of prostaglandins in the mechanism of acute pressor or the chronic hypertensive effect.Balaraman et al. (1989) in their landmark study carried out to see the pressor effect of cadmium and to postulate the mechanism for the same saw that the acute i.v administration of cadmium chloride produced marked fall in blood pressure lasting for 60-90 secs which was followed by an increase in blood pressure lasting for 10-15 minutes. The acute i.p. administration of cadmium chloride produced only a pressor response with no depressor component. They concluded that cadmium might mimic calcium ion and produce a direct contractile effect on the vascular smooth muscle because the pressor response of cadmium was blocked or reversed only by coadministration of nifedipine or verapamil which are both known to be calcium antagonists. However the possibility of alteration of fluxes of calcium ion by cadmium was not ruled out. Contrary to the above findings Toda (1973) found that cadmium inhibited the vascular response to noradrenaline. Schroeder et al. (1970) have suggested that there is a significant reduction in the pressor response to these agonists in cadmium chloride animals suggesting a decrease in vascular reactivity. Germano et al.(1984) in their study observed that in conscious rats, a single oral dose of cadmium chloride (up to 150 mg/kg) did not alter mean arterial pressure, heart rate and pressor response to phenylephrine 3, 7, and 14 days after loading. Such discrepancies in vascular reactivity to various agonists in cadmium treated animals may be due to difference in the dosage, duration of treatment, route of administration and also species. In our study it was observed that when the antioxidants alpha lipoic acid, selenium and coenzyme Q10 were supplemented with cadmium, the pressor and depressor responses to adrenaline, noradrenaline and isoprenaline were significantly reduced. This was

probably because of their cardiovascular and renal protective effect by forming inert complexes with cadmium and decreasing its toxic effects.

The electrocardiogram of the rats which were administered cadmium chloride (100 ppm) for 30 days showed changes that were suggestive of damage to the myocardium in form for S-T segment elevation which is suggestive of ischaemia, T wave inversion which is suggestive of injury to myocardium and Q wave inversion which is suggestive of infarction. When the animals administered cadmium chloride (100 ppm) was supplemented with alpha lipoic acid, selenium or coenzyme Q10 these changes were not observed and the electrocardiogram was normal suggestive of the protective effect of these antioxidants to the myocardium from the damage caused by cadmium chloride because of their antioxidant properties.

Lipids are the most susceptible targets of oxidative stress caused by free radicals. Cell membranes which are a rich source of polyunsaturated fatty acids (PUFAs) are readily attacked by oxidizing radicals and their destruction is known as lipid peroxidation. Lipid peroxidation is particularly damaging because it proceeds as a self-perpetuating reaction (Cheeseman and Slater, 1993).

In the present study, the lipid peroxidation levels in liver, kidney, lung, heart and brain were found to be increased significantly in animals exposed to 30 (except liver and heart) and 100ppm of cadmium chloride.

Malondialdehyde (MDA) is a well known end product of lipid peroxidation and has been found to increase in the liver and kidneys after Cd exposure (Shaikh et al., 1999). Reasons for lipid peroxidation after Cd exposure are not completely known, but we believe that disturbances in GSH and MT levels may allow free radicals to be "free" such that OH and O_2 radicals can attack double bonds in membrane lipids and result in an increase in lipid peroxidation. Moreover, mitochondrial respiration as the major source of ROS is promoted by lipid peroxidation and therefore enhances oxidative stress induced Cd toxicity (Karmarkar et al., 1998). In another study, Yiin et al. demonstrated that administration of Cd in various doses significantly increased thiobarbituric acid-reactive substances (TBARS), a well-known

indicator of lipid peroxidation, in rat adrenal glands that structurally contain large amounts of polyunsaturated lipids (Yiin et al., 2001). In-vitro study also reported that exposure of arachidonic acid with cadmium can also increase lipid peroxidation (Yiin, 1998).

Metal ions play a major role in propagating free radical reactions and consequently in determining the degree of free radical pathology. They are also effecting in the initiation of lipid peroxidation in a non-polar environment. Transition metal ions sch as Fe^{3+}, CO^{3+}, Ce^{4+}, Mn^{3+}, Cu^{2+} and Cd^{2+} are able to oxidize various substrates by one electron withdrawal to form cation free radicals. These radicals in turn attack the active sites of enzymes and inhibit essential enzyme function. Heavy metal ions, in particular Pb^{2+}, Cd^{2+}, Hg^{2+} and Ar^{2+} acts as an effective enzyme inhibitor.

The supplementation of alpha lipoic acid, selenium and coenzymeQ10 for 30 days significantly decreased the elevated levels of lipid peroxidation in liver, kidney, lung, heart and brain of animal exposed to 100 ppm of cadmium chloride.

Alpha-lipoic acid (ALA) is a potent antioxidant in both fat and water soluble mediums. Furthermore, its antioxidant activity extends to both its oxidized and reduced forms. DHLA is capable of directly regenerating ascorbic acid from dehydroascorbic acid and indirectly regenerating vitamin E (Scholich et al., 1989). Vitamin C (Ascorbic acid) has showed a free radical scavenging activity reducing the lipid peroxidation and restoring endogenous antioxidant levels to normal in animals exposed to heavy metals (Tondon and Singh, 2000; Patra et al., 2001; Hudecova and Ginter, 1992, Upasani, 2001)). In addition it has been reported that, vitamin E supplementation protects the animals from heavy metal induced lipid peroxidation (Patra et al., 2001; Krajcovicova-Kudlackova and Ozdin, 1995; Warren et al., 2000; Vaziri et al., 1999,). Researches have also found ALA increases intracellular glutathione (Busse et al., 1992) and coenzyme (Kagan et al., 1990) levels.

Alpha-lipoic acid (ALA) appears capable of chelating certain metals. It forms stable complexes with copper, manganese, and zinc (Sigel et al., 1978). In animal studies, it has been found to protect against arsenic poisoning (Grunert, 1960) and, in both animal and in vitro studies, ALA reduced cadmium-induced hepatotoxicity (Muller and Menzel, 1990). In vitro, ALA chelated mercury from renal slices.(Keith et al., 1997) In vitro and animal studies

suggest lipoic acid supplementation might be a beneficial component in the treatment of heavy metal toxicity, particularly toxicity involving lead, cadmium, mercury, or copper. (Gurer et al., 1999; Muller and Menzel, 1990; Muller, 1989; Sumathi et al., 1996). In one study an intraperitoneal injection of 25 mg/kg ALA given to rats for seven days was able to significantly alter the oxidative stress induced by lead toxicity. (Gurer et al., 1999). Another study demonstrated ALA at concentration of 5mM, was able to protect rat hepatocytes from cadmium toxicity (200/ [micro] M) by preventing decreases in total glutathione and increases in lipid peroxidation (Muller and Menzel, 1990). Furthermore, a study of mercury intoxication revealed an injection of 10mg/kg/day ALA in rats inoculated with 1 mg/kg/day mercuric chloride prevented damage to nerve tissue caused by lipid peroxidation (Anuradha and Varalakshmi, 1999).ALA appears to improve tissue redox status in metal toxicity and during chelation with dithiol compounds, including dimercaptosuccinic acid (DMSA) (Pande and Flora, 2002).

Selenium is a potent antioxidant. Elemental selenium is practically inert nutritionally and toxicologically. On the other hand, different inorganic or organic compounds of selenium can be converted in a mammalian organism into biologically active form. It is an integral part of the body's natural antioxidant-glutathione peroxidase system, and at times in partnership with Vitamin A protects against cancer and prevents lipid peroxidation. Selenium treatment was postulated to protect the kidney tissues against the toxicity of cadmium since it reduced MDA levels and increased the activities of antioxidant enzymes in these tissues (El-Sharaky et al., 2007).

Coenzyme Q10 is a component of the oxidative phosphorylation in the mitochondria, which converts the energy in carbohydrates and fatty acids into ATP to drive cellular machinery (Ernsteer and Dallner, 1995). In addition to assisting electron transfer during oxidative phosphorylation, CoQ10 inhibits certain enzymes involved in the formation of free radicals and thus attenuates oxidative stress. It can also function beneficially by virtue of its free radical scavenging and inhibition of phospholipase activity (Stocker and Frei, 1991). CoQ10 treatment also significantly decreased lipid peroxidation and increased SOD, catalase and reduced glutathione levels in the liver tissue homogenate, which might be the result of a decrease in oxidative stress. In coronary artery disease patients, CoQ10 supplements were

also associated with a significant reduction in thiobarbituric acid reactive substances, malondialdehyde and diene conjugates (Singh et al., 1999). Furthermore CoQ10 reduced lipid peroxidation and increased antioxidants such as glutathione in the liver homogenates of the treated animals. (Modi et al., 2007).

In this study, the superoxide dismutase and catalase levels were significantly decreased in all the organs of the animals exposed to 30(except heart and brain) and 100 ppm of cadmium chloride. This may be attributed to the higher amounts of superoxide radical and hydrogen peroxide in the organs due to the oxidative damage caused by cadmium. The levels of reduced glutathione in liver, kidney lung and heart were decreased significantly after thirty days exposure to cadmium chloride 100 ppm. The reduction of these endogenous antioxidants on administration of cadmium and other heavy metals has also been reported in other studies with an increase in lipid peroxidation (El-Missiry, 2000; Thevenod, 2000; Karmarkar et al., 1998).

Oxidative stress is caused by exposure to these reactive oxygen intermediates, such as superoxide anion (O_2), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH) that can damage proteins, nucleic acids and cell membranes Increasing evidence suggests that the cumulative damage caused by reactive oxygen species contributes to numerous diseases(Aruoma and Halliwell, 1998). Oxidative stress is an unavoidable by product of the aerobic lifestyle because of superoxide and hydrogen peroxide radicals, which are formed wherever molecular oxygen chemically oxidizes electron carriers. This superoxide concentration is tolerable, about half what is necessary to diminish the activities of vulnerable enzyme and inhibit cell growth. Thus the defences in the cell are calibrated to just avoid toxicity from endogenous oxidants. These defenses are inadequate, however, if the rates of intracellular O_2 and H_2O_2 formation are accelerated. O_2 and H_2O_2 have different chemical reactivity and generate distinct type of damage inside cells. 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). Catalase has been reported to be responsible for the detoxification of hydrogen peroxide (Brenner and Alison, 1953). Catalase may function to protect the cells against the onslaught of horrendous

amounts of hydrogen peroxide. Catalase deficient organisms are more rapidly killed by hydrogen peroxide (MamEation, 1990).

Metal-induced alterations in antioxidant enzyme activities have been extensively studied over the years. It has been reported that especially CAT and SOD, two major antioxidant enzymes are affected by Cd. SOD activity was found to be inhibited by administration of cadmium acetate to liver and kidneys *in vitro*, as well as *in vivo*.

Reduced glutathione is another enzyme which is a protective molecule against chemical induced cytotoxicity (Orrenius and Moldeus, 1984). It helps in removing hydrogen peroxide by converting it to water. This enzyme converts hydrogen peroxide to water by using it to oxidize reduced glutathione (GSH) to oxidized glutathione (GSSG). Glutathione is tripeptide of glutamate, cysteine and glycine. It is found at a high (millimolar) concentration in most aerobic cells. As an antioxidant, glutathione plays a critical role in reducing hydrogen peroxide to water by enzyme glutathione peroxidase. . Alterations in GSH levels have been observed in Cd toxicity, and some studies reported an increase in GSH levels after Cd exposure (Rana and Boora, 1992; Kamiyama et al., 1995; Rana and Verma, 1996; Shaikh et al., 1999). A few studies reported GSH depletion in tissues (Karmarkar et al., 1998, Shibasaki et al., 1996).

Co-administration of alpha lipoic acid, selenium and coenzyme Q10 restored the levels of superoxide dismutase, catalase and reduced glutathione to normal in all the organs indicating their powerful antioxidant activity.

Alpha lipoic acid increases the levels of vitamin E and vitamin C which in turn has a modulatory effect on cadmium toxicity due to their lipid peroxidation chain breaking reactions (Packer, 1995). Selenium supplementation in acute cadmium toxicity has been known to decrease lipid peroxidation in rat studies and has also been shown to increase the production of glutathione S-transferase and glutathione peroxidase in rhesus monkeys. Similar results elevations of glutathione peroxidase and decreased whole body and renal burden of cadmium were found in rats given daily selenium supplementation of 350 μg/kg body weight. CoQ10 inhibits certain enzymes involved in the formation of free radicals and

thus attenuates oxidative stress. It can also function beneficially by virtue of its free radical scavenging and inhibition of phospholipase activity. (Stocker and Frei, 1991).

ATPases are a class of enzymes that catalyze the decomposition of adenosine triphosphate (ATP) into adenosine diphosphate (ADP) and a free phosphate ion. This dephosphorylation reaction releases energy, which the enzyme (in most cases) harnesses to drive other chemical reactions that would not otherwise occur. This process is widely used in all forms of life. They also play a very important role in the active transport of cations when activated (Luly et al., 1972). The biological membranes like erythrocytes exhibit two Ca^{++}-ATPases components, which differ with respect to their affinities for the calcium ions. These ATPases require high amounts of calcium (10mM) for their activity (Hjerken and Pan, 1983). Any imbalance in the calcium ions leads to the change in the activtivity of Ca^{++}-ATPases.
Cadmium is reported to interfere with the activity of calcium in the body, thereby affecting the levels of these ATPases. Mg^{++}-ATPase is referred to as the basic ATPase and its presence has been reported mainly in the plasma membrane, liver, and kidney etc. (Ohinishi et al., 1982). The present study showed a significant decrease in the levels of all the three ATPases in the organs of the animals exposed to 100 ppm of cadmium chloride. The same results are also been seen in other studies with heavy metals like lead (Jarrar and Mahmoud, 2000; Upasani and Balaraman, 2001a). The decrease in ATPases may also be observed as the heavy metals interfere with and cause deficiency of nutritionally essential metals (Goyer, 1973) eg cadmium competes with zinc for the binding sites on metallothionein. ATPases are a part of the cell membrane which is rich in lipids especially phospholipids hence the changes in ATPases may also be due to the changes in levels of cholesterol, phospholipids and triglycerides. Alpha lipoic acid, coenzyme Q10 and selenium by their ability to scavenge free radicals and reduce the oxidative stress by cadmium regularizes the lipid levels thereby preventing the inactivation of –SH group and stabilizing the membrane bound enzymes. Thus the levels of the ATPases were restored to normal in the rats when alpha lipoic acid, selenium and coenzyme Q10 was supplemented along with cadmium chloride.

Lipids are the most susceptible targets of oxidative stress caused by free radicals like cadmium in form of prooxidant and peroxidative damage to membrane fagility and permeability. The present study demonstrated a significant increase in the cholesterol,

triglyceride and phospholipids levels in liver, kidney, lung, heart and brain in animals exposed to cadmium chloride (100 ppm). This may be attributed to the damage of cell membranes by cadmium which is a rich source of polyunsaturated fatty acids (PUFAs). Lipid peroxidation is particularly damaging because it proceeds as a self-perpetuating reaction (Cheeseman and Slater, 1993) consequent to free radical production in cell because it is a destructive chain-reaction that can directly damage the structure of the membranes and indirectly damage other cell components by the production of reactive aldehydes. It is reported that lead and cadmium exposure causes dose dependant increase in the cholesterol, triglyceride and phospholipids levels in the organs (Honchel et al., 1991; Lawton and Donaldson, 1991; Skoczynska and Smolik, 1994). The increase in cholesterol, triglyceride and phospholipids is due to altered lipid metabolism in the tissues. 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 bi layers of cell membranes. This oxidative modification of lipids which starts during the stress by free radicals is known to be protected by various antioxidants (Retsky et al., 1993). In the present study, alpha lipoic acid, coenzyme Q10 and selenium have been shown to normalize the cholesterol, triglyceride and phospholipids levels in liver, kidney, lung, heart and brain in animals exposed to cadmium chloride (100 ppm). Alpha-lipoic acid is a potent antioxidant in both fat and water soluble mediums. Furthermore, its antioxidant activity extends to both its oxidized and reduced forms. DHLA is capable of directly regenerating ascorbic acid from dehydroascorbic acid and indirectly regenerating vitamin E (Scholich et al., 1989). Vitamin E is a potent scavenger of free radicals generated by oxidative damage. It is also lipid soluble which plays an important role in breaking the chain reaction of lipid peroxidation. Vitamin C is a strong electron donor and it rapidly oxidizes leading to transient formation of dehydro-L-ascorbic acid, and utilizes the oxygen formed in the organ; thereby it protects against oxidative modification of lipids. CoQ10 inhibits certain enzymes involved in the formation of free radicals and thus attenuates oxidative stress. It can also function beneficially by virtue of its free radical scavenging and inhibition of phospholipase activity (Stocker and Frei, 1991). The ability of selenium to decrease the tissue burden of cadmium has been repeated in other animal studies. Acute toxicity studies have found that, as a result of dosing selenium and cadmium at the same

time, organ tissue levels of both metals increased and the toxic burden of cadmium decreased, possibly as a result of inert nature of the cadmium/selenium complex.

Transaminases are mainly liver enzymes but widely distributed in serum and other tissues which are involved in the intermolecular transfer of an amino group from a donor α- amino acid to an acceptor α- keto acid without formation of ammonia. The present study showed a significant increase in the levels of glutamate pyruvate transaminase (GPT) and glutamate oxaloacetate transaminase (GOT) in serum of the animals treated with cadmium chloride. Previous studies have also demonstrated the same in heavy metals. (Columbano et al., 1983; Dhar et al., 1980; Lupo et al., 1986). The increase in these liver enzymes is attributed to injury to the hepatocytes due to the lipid peroxidation by oxygen metabolites generated due to the oxidative stress by free radicals. The leakage of these enzymes from damaged membrane is very high and appears in serum rapidly (Pappas, 1986). Alpha-lipoic acid appears capable of chelating certain metals. It forms stable complexes with copper, manganese, and zinc (Sigel et al., 1978). In animal studies, it has been found to protect against arsenic poisoning (Grunert, 1960) and, in both animal and in vitro studies, ALA reduced cadmium-induced hepatotoxicity (Muller and Menzel, 1990). Selenium is an integral part of the body's natural antioxidant-glutathione peroxidase system, and at times in partnership with Vitamin A protects against cancer and prevents lipid peroxidation. Selenium may influence the synthesis of glutathione peroxidase, which facilitates the lowering of tissue peroxide levels in the body by destroying hydrogen peroxide. Acute toxicity studies have found that, as a result of dosing selenium and cadmium at the same time, organ tissue levels of both metals increased and the toxic burden of cadmium decreased, possibly as a result of inert nature of the cadmium/selenium complex. CoQ10 inhibits certain enzymes involved in the formation of free radicals and thus attenuates oxidative stress. It can also function beneficially by virtue of its free radical scavenging and inhibition of phospholipase activity (Stocker and Frei, 1991). Thus by the above mechanisms alpha lipoic acid, selenium and coenzyme Q10 protected the organs especially the liver from damage due to the free radicals produced by cadmium and restored the altered levels of transaminases GPT and GOT to normal levels.

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 level of alkaline phosphatase in the disease related to bone, liver and biliary tract. Most of the increase may follow as a result of cholestasis induced by any toxicant including cadmium as a result of hepatocellular toxicity caused by the death of the hepatocytes due to lipid peroxidation by the oxidative enzymes. Epidemiological studies have found a positive correlation with elevated urinary cadmium levels and increased urinary calcium loss and elevated serum alkaline phosphatase levels. Studies have also found correlations between cadmium-induced renal tubular damage and bone loss. The mechanisms behind cadmium and bone loss are related to renal tubular cell damage that results in elevated levels of urinary calcium and lowered levels of 1,25 dihydroxy-cholecalciferol, a consistent finding in women environmentally exposed to significant levels of cadmium. Lower levels of vitamin D3 alter calcium homeostasis by decreasing absorption of calcium in the gut and altering deposition in bone leading to osteoporosis (Staessen et al., 1999; Alfven et al., 2000, 2002; Honda et al., 2003a; Aoshima et al., 2003; Jin et al., 2004). Probably due to the above reasons we noted a significant increase in the levels of alkaline phosphatase in the serum of animals treated with cadmium chloride. Our finding was consistent with that noted in previous studies (Othman and El-Missiry, 1998; Upasani, 2001). Alpha lipoic acid, selenium and coenzyme Q10 down regulated the elevated levels of alkaline phosphatase in animals exposed to cadmium by their hepatoprotective action and by restoring the levels of membrane bound enzymes, lipids and correcting the cytosolic disturbances in cadmium exposed rats.

All these elevated levels of transaminases and phosphatases suggests that normal metabolizing capacity is diminished in liver and other organs by cadmium intoxication causing oxidative stress leading to lipid peroxidation.Treatment with alpha lipoic acid, selenium and coenzyme Q10 restored this level to normal.

Lactate dehydrogenase is of clinical significance in various diseases. As it is elevated in diseases like myocardial infarction, hepatitis and certain types of cancers it is used as a diagnostic tool. 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. The free radicals generated by heavy metals like cadmium are also electron transfer agents and compete with lactate dehydrogenase for the same. Cadmium has also been seen to cause hepatotoxicity and infarction of the myocardium. Thus in animals given cadmium chloride we found an increase in the serum levels of lactate dehydrogenase which was related to the dose of cadmium. Lactate dehydrogenase levels have also been found to be elevated in previous studies of heavy metal toxicity (Colderon-Salinas et al., 1993; Sakaguchi et al., 1982). Alpha lipoic acid, selenium and coenzyme Q10 restored the levels of lactate dehydrogenase to normal in serum indicating their antioxidant properties which helped in protecting the organs from the oxidative damage induced by cadmium by interfering in the electron transfer stage of NADH oxidation.

Serum bilirubin levels are found to increase in liver disorders like hepatitis. In liver the main changes are in form of fatty degeneration and inflammatory infiltration in animals fed with cadmium chloride. The presence of inflammatory cells in form of neutrophils and lymphocytes indicates that an active process of destruction of the liver cells is going on. This inflammatory response and its subsequent repair have been shown to be mediated by cytokines which is mediated by oxidative stress (Dong et al., 1998: Ledda-columbano et al., 1983; Kayama et al., 1995; Lawton and Donaldson 1991). This damage to the hepatic cells by cadmium is probably the reason why serum bilirubin levels were found elevated in animals fed with cadmium chloride. Alpha lipoic acid, coenzyme Q10 and selenium by their antioxidant properties have a protective effect on the liver and prevent the damage caused by cadmium when supplemented with it. Thus in the animals where these antioxidantts were supplemented along with cadmium the serum bilirubin levels remained near normal.

The protein levels in serum were significantly reduced in animals exposed to cadmium 100 ppm for 30 days. This can be attributed to the catabolic effects of the toxic metal due to cell breakdown caused by oxidative damage. Alpha lipoic acid, selenium and coenzyme Q10 by their anabolic effect significantly restored the decreased levels of proteins in serum to normal.

The cholesterol levels in serum were decreased significantly and correspondingly the levels of serum triglycerides were significantly increased in animals exposed to 100 ppm of