Discussion
Chapter 5: Discussion

Cadmium is a ubiquitous toxic metal that may induce oxidative damage by disturbing the prooxidant-antioxidant balance in the tissues. It promotes an early oxidative stress and afterward contributes to the development of serious pathological conditions because of its long retention in some tissues (Grosicki et al., 2002). Various mechanisms have been suggested to be responsible for the cadmium toxicity. One of these mechanisms is related to sulfhydryl (-SH) groups, that cadmium toxicity can cause oxidative stress by interaction with (-SH) groups of major intracellular defender glutathione. Another possible mechanism of cadmium intoxication leads to lipid peroxidation (LPO) after consequent decrease of GSH. Various studies have shown that cadmium toxicity seems to be crucially mediated by the formation of reactive oxygen species (Flora et al., 2008). However, by administration of dietary nutrients, the deleterious effects of Cd affecting antioxidant system and LPO tend to reach control value. These antioxidants including dietary nutrients exert protective effects on the affected antioxidant defense system. This defense system includes the enzymes, glutathione peroxidase, glutathione-S-transferase, superoxide dismutase, catalase as well as glutathione that normally protects against free radical toxicity (Pourahmad et al., 2003). The damaging action of cadmium on the soft tissues during its exposure have been extensively studied (Jihen et al., 2008). The hypothesis that the supplementation of dietary nutrients either individual or in combination offers beneficial effects in reversing Cd induced oxidative stress was examined in the present study.

Changes in the body weight and organ/body weight ratio have been used as indices of chemical toxicity. The significant alterations observed in these parameters in Cd exposed rats are indicator of toxicity which exhibits a decreasing trend in the body weight and increase in organ/body weight ratio throughout the course of experiment. There was a significant improvement in these indices in rats, when simultaneously administered dietary nutrients alone or in combination in Cd exposed animals. This reversal of the effect of Cd on organ weight expressed as organ/body weight ratio by the nutrients supplementation provided a strong indication that supplementation with dietary
nutrients was helpful in ameliorating the effect of cadmium toxicity. The present results
find support from the observations of Asagba et al., (2007), Jurczuk et al., (2004),
Horiguchi et al., (1996). They reported that rats given cadmium for two or four weeks,
had statistically significant alterations (p<0.05) viz lower final body weight compared to
control rats receiving saline only. The same results were observed by Borde et al., (2008)
in rats demonstrating intoxication effect of Cd.

Our results indicated a significant increase in the toxic metal level in the liver,
kidney and blood with higher amount in the kidney which was evident from the data
showing maximum accumulation of cadmium after 21 days (Table 14 & 39). Our
observations are in agreement with the findings of previous workers (Kim et al., 1998;
Ongjanovic et al., 2008).

In fact, it has been reported that, after its absorption, Cd is taken up by the
hepatocytes, and then from the liver it circulates in blood bound to metallothionein (MT)
(Nordberg and Nordberg, 1987). The Cd–metallothionein complex (CdMT), because of
its small molecular size, gets easily filtered through the glomerular membrane and taken
up by renal tubular cells. MT is then catabolized releasing Cd ions in the cytoplasm
where they induce synthesis of new MT molecules. This, in turn, binds and retains Cd in
the kidney for a long period of time (Nordberg et al., 1994).

Through this study, we have also investigated some effects of oral intake of
dietary nutrients on Cd accumulation in the liver, kidney and blood. Se was found to
have protective effect by significantly decreased Cd content in the liver, kidney and
blood. According to the available data, it seems that Se can reduce Cd accumulation in
organs (Jamba et al., 2000; Stajn et al., 1997). Among the possible mechanisms
explaining the protective effect of Se on Cd toxicity, antagonism to Cd- induced DNA
damage and apoptosis (Yu and Chen, 2004) and amelioration of antioxidant system (El-
Sharaky et al., 2007; Newairy et al., 2007). In Zn co-treated animals, although we have
found a significant decrease in renal and hepatic Cd levels. We noticed an improvement
in the Cd-induced damage in the liver, but above all, a complete prevention of the renal
structural changes was observed. Our findings are in agreement with the work of
Jacquiller et al., (2006) who reported that the effect of co-treatment with Zn during Cd
administration completely prevented the changes in the renal function produced by the toxic metal in the rat, even though they did not find any significant difference in the renal Cd content. There is considerable information available in the literature regarding the protective effect of Zn against the cellular toxicity caused by Cd. Previous studies (Bonner et al., 1981) concluded that Zn protection is perhaps due to redistribution of Cd in the organism since Zn is able to induce synthesis of MT in the liver and kidney. In recent studies, Zn has been demonstrated to play an active role in preventing oxidative stress, apoptosis and necrosis induced by Cd (Jemai et al., 2007).

In addition to Cd, Cu and Zn levels were also determined as they are essential elements for the maintenance of life and health. In the rats treated with Cd there was a significant decrease in the levels of these trace elements as compared to control. This may be due to interference of cadmium on absorption and transport of these trace elements, which would have resulted in the depletion of these metals in this group of rats. Cd may inhibit zinc activities at many stages, interfering with absorption, distribution and transport of zinc into cells or into several intracellular structures (Nath et al., 1993; Bin et al., 1994). Co-administration of dietary nutrients either individually or in combination normalized the levels of these trace elements in blood and tissue as compared to Cd intoxicated rats (Aughey et al., 1984; Dudley et al., 1985; Mitusumori et al., 1998; Jamba et al., 1997).

The present study reveals that rats exposed to Cd alone showed significant reduction in haematological variables such as Hb, PCV and GSH. The haemoglobin concentration showed a decreasing trend through day 7, 14 and 21 on the exposure of CdCl₂ as compared to control rats receiving saline only (Table 6 & 17-19). The decrease in hematological parameters (Hb, GSH and PCV) is in agreement with the work of Karmakar et al., (2000) who showed that cadmium chloride also caused changes in the blood indices of rats. The results obtained in present study show that treatment with Cd induced anemia in rats. It is well known that the presence of cadmium in the rats decreased the level of iron in the blood (Kostic et al., 1993a) which was responsible for declined Hb concentration. The reduction in Hb content may be due to increased rate of either destruction or reduction in the rate of formation of erythrocytes. In addition, the
reduction in the blood parameters may be attributed to hyperactivity of bone marrow leading to production of red blood cells with impaired integrity that are unstable in the circulation (Tung et al., 1975).

Hematocrit is another haematological variable directly related to Hb content and variations in Hb content are directly manifested in PCV value. There may be three possible causes for the decrease of the haematocrit (PCV) during the stress: increase on the volume of the plasma, loss of water in the erythrocyte and haemolysis of erythrocytes in the blood stream. The response to stress is characterized by hormone change (catecholamine and corticosteroid) that induced alteration on the haematological parameter. Shukla et al., (1996) and Hamada et al., (1998) have also confirmed the similar reason for decreasing packed cell volume in rats.

The results of our experiments showed that, in animals exposed to Cd, the GSH was significantly decreased as compared to control rats. The reduction in activity of GSH might be due to its consumption in the scavenging free radicals generated by Cd (Bagchi et al., 1996; Nigam et al., 1999). Also, GSH may be consumed in the detoxification of Cd. In fact, it has been reported that the sulphydryl group of cysteine moiety of glutathione has a high affinity for metals such as Cd, forming thermodynamically stable mercaptides complexes which are inert and excreted via the bile (Xiao et al., 2002; Mohanpuria et al., 2007).

El-Demerdash et al., (2004) did not find any significant change in haematological parameters, while ameliorated the harmful effect of CdCl₂ after administration of Vit-E (100mg/kg). This may be due to the higher dose of CdCl₂ (5 mg/kg for 30 days selected for their experiment). On the other hand, there are many studies which show that supplementation of Vit-E (100 mg/ kg) (Ognjanovic et al., 2001; Bansal et al., 2005; Nemnich et al., 2007; Kanter et al., 2009), leads to elevation in haemoglobin and hematocrit of rats co-exposed to varying dose of CdCl₂ ranging from almost 0.5 mg to 4 mg. Tandon et al., (2003) and Chichovska et al., (2006), who showed a significant increase in the GSH level after supplementation of Zn (10 mg/kg) and NAC (5 nmol/4 ml/ kg) which maintained the levels of antioxidants, membrane-bound enzymes and the
activities of antioxidant enzymes near normal levels, thus emphasizing their effects as antioxidants (Valko et al., 2006).

The activity of AST and ALT enzymes in blood serum may also be used as stress indicator. The significant changes in the activities of these enzymes in blood serum indicate tissue impairment caused by stress (Sarkar et al., 1998). In the present study there were significant changes in AST and ALT activities in serum of rats exposed to Cd compared to the control group. The increase in concentration of AST and ALT in blood serum indicates impairment of parenchymatous organ such as liver. Additionally, elevated serum levels of these variables may be due to hepatocellular necrosis (Fig 36 & 37) which causes increase in the permeability of the cell membrane resulting the release of transaminases in the blood stream. The increase in plasma AST and ALT activities indicates an active transamination of amino acids and involvement of keto acids that are probably fed into tricarboxylic acid cycle (TCA) for oxidation. The increase in the liver AST and ALT activities may be due to liver dysfunction and disturbance in the synthesis of these enzymes. Therefore, the increase in the activities of AST and ALT in plasma indicating the hepatotoxic effect of cadmium chloride (Table 20 & 21) and is mainly due to the leakage of these enzymes from the liver cytosol into the blood stream (Vandenberghe, 1995; Rana et al., 1996; Shaikh et al., 1999; Sharma et al., 2002). Administration of dietary nutrients was beneficial in reversing the levels of ALT and AST very close to the normal. These results are in agreement with the findings of Konar et al., (2007) who noticed protective effects of various combinations of melatonin, Vit-E and Se in Cd exposed rats. The application of dietary nutrients and their combination restored the control value in different serum contents tested during different time of exposure. It may testify the possibility of dietary nutrients arresting cadmium free radicals, consequently decreasing the damage caused by this metal.

Previous studies have demonstrated that Cd intoxication may also result in renal tubule damage specifically glomerular filtration impairment (Shibutani et al., 2001). This may account for the increase of urea and creatinine concentration in the animals receiving cadmium chloride in the present study. The damaging effects of cadmium on kidneys have also been described by other authors (Jarup et al., 2000; Jarup et al., 2002).
Present results coincide with earlier reports that a significant increase in serum urea and creatinine or decrease in potassium levels (Ali et al., 1992; Erdem et al., 2000; Kopple et al., 2002; Atessahin et al., 2005; Harlalka et al., 2007) might indicate a nephrotoxic condition in Cd-treated rats and may be due to kidney damage caused by the enhanced generation of ROS. These findings can be correlated well with the renal histological examination, which revealed renal tubular necrosis and interstitial nephritis (Plate 64-66). This is supported by the findings of Szilagyi et al., (1994) who reported that alterations in serum urea may be related to metabolic disturbances (e.g. renal function, cation-anion balance). Katyal et al., (1997) reported that, the increase in urea concentrations in plasma of animals treated with Al may be due to its effect on kidney function, as urea is the end product of protein catabolism, and/or referred to kidney dysfunction.

In the present study, activity of creatinine was increased significantly in the kidney of Cd exposed rats. This may be due to the damage of large number of nephrons. Only renal dysfunction changes the results, however, the serum creatinine level will not rise until at least half of the kidney’s nephron are destroyed or damaged. Because creatinine rise and fall more slowly than urea levels, CRE levels are often preferred to monitor renal function on a long term exposure (Obiadh et al., 2009).

Our findings show that 2.0 mg/kg CdCl₂ administration for 21 days significantly increased levels of serum AST, ALT, URE and CRE. These findings are similar to the findings put forth by the previous researchers (Chakraborty et al., 2009; Kumar et al., 2010). Several researchers reported that various dietary nutrients like Vit-E (Nemmiche et al., 2007), Se (Alhazza et al., 2008), NAC (Tandon et al., 2003) and melatonin (Konar et al., 2007) were effective against haematological toxicity caused by cadmium chloride. Moreover, the results of current study demonstrated that *Spirulina platensis* itself significantly improved the damages of hepatocytes and renal tissues specifically glomerulus filtration for they normalized the activities of these hepato-renal markers (Amin et al., 2007; Sabina et al., 2009).

In our investigation a decrease in the concentration of oxidation stress biomarkers (SOD and GPx) was observed in animals receiving CdCl₂ and dietary nutrients in relation to those receiving only CdCl₂. These enzymes have the role in
decomposing free radical compounds e.g superoxide dismutase (catalyses the reduction of superoxide anions to hydrogen peroxide), glutathione peroxidase (catalyses the reduction of hydrogen peroxide to water in the presence of glutathione). The same results were observed by Gupta et al., (2005) in rats indicating the decrease in SOD activity. This could be attributed to an enhanced superoxide production during metal metabolism. The accumulation of Cd and SOD inhibition was highest in liver followed by kidneys, indicating direct effect of Cd on SOD activity. This suggests the role of free radicals in causing cellular damage during long-term Cd exposure (Patra et al. 1999). SOD accelerates the dismutation of superoxide ($\text{O}_2^-$) to $\text{H}_2\text{O}_2$, providing primary defense, as it prevents further generation of free radicals. Superoxide radicals are constantly generated in the body tissues and failure in their immediate removal can initiate a damaging effect on the poly-unsaturated fatty acids and structural proteins of plasma membrane. It appears that Cd inhibits SOD activity by directly interacting with the SOD molecules in tissues. SOD is a metallic enzyme, depending on its subcellular origin contains Cu/Zn or Mn. SOD inhibition by cadmium might have interacted with polyunsaturated fatty acids and produced a higher concentration of lipid peroxides by the process of peroxidation. Few studies have shown that Cd inhibit the activity of majority of enzymes involving in antioxidant defense system, resulting high production of free radicals causing lipid peroxidation and cell membrane destruction. The decreased activity of GPx by Cd intoxication (Table 26 & 27) could be due to competition of Cd-metallothioneins and GPx for sulfur containing amino acids (Casalino et al., 2002). The similar results were observed by Waisberg et al., (2003). Whereas, Wang et al., (1997) observed a decrease in the level of GSH with increased lipid peroxidation (LPO) resulting in reduced activity of GPx during arsenic exposure.

Several researchers reported that various antioxidants like Vit-E, Se, NAC and melatonin were effective against oxidative stress caused by cadmium. Shaikh et al. (1999) reported that antioxidants such as vitamin E or N-acetyl cysteine were effective against hepato-toxicity or renal toxicity caused by cadmium and reduced oxidative stress. Stajn et al., (1997) noted that 200 ppm Cd and 0.1 ppm Se administration to rats via the oral route, for a month, reduced lipid peroxidation caused by Cd and increased SOD and GPx activity. Similar protection against Cd induced oxidative stress was also
noticed following treatment with Zn and Se individually (Xiao et al., 2002) and also in combination (Jihen et al., 2008). Additionally, arsenic intoxicated rats following supplementation with methionine and cysteine have also been obtained to produce tissue specific protection from oxidative damage in the rats with more reactivity of methionine in the liver than cysteine, which may be due to the fact that former is readily taken up by the hepatocytes for the synthesis of glutathione, a low molecular mass antioxidant and thereby protects this organ from impending damage by free radicals (Reed and Orrenius, 1997). Our findings associated with above mentioned variables obtained after supplementation of selected dietary nutrients are parallel to these results.

According to present data, the activity of alkaline phosphatase and total protein significantly decreased in the Cd exposed group compared to the control rats. Reduction in ALP activity may be due to destruction of hepatocyte inhibition of bile production or decrease of alkaline phosphatase by failure of secretory function of liver cells. The decline in total protein may be due to utilization of protein caused abnormalities in fat deposit cell of liver following disturbance in the protein metabolism. Additionally, increased production of free radicals, which initiates lipid peroxidation leads to cellular damage. Similarly, some authors have shown that chronic exposure to Cd decreased the level of ALP and total protein in the liver and kidney of rats (Eriyamremu et al., 2008; Pal et al., 2010). Whereas, Sarkar et al., (1998) and Obianime et al., (2009), showed that antioxidant administration to rats concomitant to their intoxication with Cd although normalized ALP and total protein levels but also caused a significant decline in the liver and kidney peroxide formed within 24 hrs.

However, selenium treatment provides significant protection against Cd toxicity in rats. Recently Alhazza et al., (2008) showed the beneficial influence of Se alone reducing the harmful effects of CdCl₂. Our results showed that the nutritional antioxidants i.e., Se (Ognjanovic et al., 2008), NAC (Tandon et al., 2003), Vit-E (Kanter et al., 2009), Zn (Chichovska et al., 2006), melatonin (Kim et al., 1998) and methionine (Patra et al., 2004) ameliorated oxidative stress and loss of cellular antioxidants and suggested that dietary nutrients protect hepatic and renal toxicity induced by cadmium intoxication.
In our study, exposure to Cd was shown to increase LP concentration in the tissues of rats. This can be explained by the excessive production of free radicals. Cd ions are strong inducers of production of the superoxide anion radical (\(O_2^-\)) and of hydrogen peroxide (\(H_2O_2\)), leading to oxidative stress (Jurczuk et al., 2004). In vivo, Cd increases the formation of TBARS in lungs, liver and brain, and also urinary excretion of malondialdehyde (MDA), products of lipid peroxidation (Bagchi et al., 1996; El-Maraghy et al., 2001). The blood concentration of LP reflects not only the processes of lipid peroxidation occurring in red blood cells, but also in the whole organism. In fact, El-Demerdash et al. (2004) and Kara et al. (2005) observed a positive relationship between LP concentration in the blood and LP concentrations in the liver, kidney, testes and brain of rats exposed to Cd. Lipid peroxidation is one of the main manifestations of oxidative damage, playing an important role in the toxicity of many xenobiotics (Stohs and Bagchi, 1995; Anane and Creppy, 2001). Our data (Table 32 & 33) confirmed that chronic intoxication with cadmium causes a significant increase of lipid peroxide concentration in liver and kidneys of rats measured in terms of thiobarbituric acid reactive substance (TBARS) which is in accordance with Gupta et al., (2005) and Newairy et al., (2007) since it causes lipid peroxidation in numerous tissues both in vivo and in vitro experiments. Cadmium stimulates formation of ROS, including superoxide anion radical, hydrogen peroxide and probably hydroxyl radical. As a consequence enhanced LP, DNA damage, altered calcium and sulphydryl homeostasis, as well as marked disturbance in AOS occurs. The present findings are also affirmation to the findings of Casalino et al., (2002) in rats after cadmium intoxication. Further, Hassan et al., (2007) reported similar results in which SOD and GPx activities were inhibited by the cadmium exposure in rats.

Co-treatment with dietary nutrients was significantly effective in the prevention of oxidative damage induced by Cd, which resulted in significantly lower LP concentration in the liver and kidneys. This explains the important role of dietary nutrients in preventing lipid peroxidation and in protection of integrity and functioning of tissues and cells. Thiols such as GSH play a pivotal role in protecting cells against ROS, while SOD, catalase and GPx being the most important enzymes against toxic effects of oxygen metabolism (Pande and Flora, 2002; Saxena and Flora, 2004). It was
observed that *Spirulina platensis* when given in combination with CdCl₂ significantly elevated antioxidant enzyme activity and declined lipid peroxidation thus, reducing the Cd toxicity. Present findings are in agreement with the findings of Kumar et al., (2005) and Jeyaprakash et al., (2005).

Dietary nutrients administration shows the reduction of LP observed in liver and kidney related to the increase of SOD and GPx activity. Superoxide dismutase activity was increased as SOD converts superoxide anion into H₂O₂ and O₂, while GPx reduced H₂O₂ to H₂O resulting in the detoxification of free radicals. Thus, elevation of SOD and GPx activity may contribute to decrease of LP. This may also be due to the effect of dietary nutrients inducing reduction of energy expenditure, consequently leading to lowered ROS. Reduced LP products suggest decreased formation of fatty acid epoxide and subsequent free radical damage to cellular macromolecules. In addition, dietary nutrients may lead to reduced mitochondrial oxyradical production and/or increased expression of cytoprotective stress protein, which may suppress oxyradical production and stabilize cellular homeostasis. SOD appears as an important enzyme for the prevention of aging and mutation by oxidative stresses and hazardous effects from environmental factors (Seo et al., 1997). GPx also plays an important role in protecting mammalian cells against oxidative damage (Mates, 2000). Thus, the beneficial effects of dietary nutrients on these enzymes may promote the capacity of liver and kidney to protect against toxic actions of ROS, maintaining normal function.

In the present work, the structure of both organs was evaluated on the basis of histopathological findings (Plate 30 & 63). Cd accumulation in the liver and the kidney resulted in severe structural changes in these organs (Plate 33 & 66). Other investigators (Aughey et al., 1984; Brzoska et al., 2003; Koyu et al., 2006; Nakazato et al., 2007) have noted similar or more pronounced changes in the hepatic and renal tissues under Cd effect. In fact, it has been suggested that Cd disturbs membrane integrity, generates reactive oxygen species and involves cytotoxic and inflammatory mediators, in the liver and kidney (Kayama et al., 1995). During acute and chronic exposure, cadmium-induced necrosis and cellular damage in kidney and liver. Yiin et al., (1999b) reported that kidney is a critical target organ following cadmium exposure. Renal metabolism is perturbed in both acute and chronic exposure. Adaptive mechanisms counteract renal
tubular acidosis during chronic, but not following, acute exposure. Following chronic exposure, the kidney also shows an alteration in lipid content, possibly caused by mitochondrial proliferation (Griffin et al., 2001). The renal effects are generally considered to be mainly tubular. Chronic cadmium exposition caused a nephropathy with peculiar damage of the renal proximal tubule. Atrophy and degeneration of proximal tubules with vesiculation of tubular cells were striking lesions seen. The present alterations are also in affirmation to the findings of Brzoska et al., 2003. Cadmium exposure has been shown to cause large cytoplasmic vacuoles containing membranous material in proximal tubular lining cells. Light microscopy showed focal areas of necrosis and interstitial fibrosis within the renal cortex. These findings are associated with renal cadmium levels (Scott et al., 1977; Goyer et al., 1989).

Liver is well known as the major organ of chemical toxicity due to a high dose of CdCl₂ administration (Goering and Klaassen, 1984). Most of the cadmium absorbed during chronic exposure is initially trapped in the liver and gradually redistributed to the other organs, mainly to kidney (Shaikh et al., 1999). In the liver, cadmium is stored as metallothionein (MT) complex (Webb, 1986). This protein has a rapid turnover, but the liver has very high synthetic capacity for MT thus traps cadmium efficiently. In the present examination, after 21 days of cadmium administration the changes in the hepatic tissue indicated that excess of cadmium, which could not be detoxified with induced MT complex consequently causing cadmium toxicity. The cadmium toxicity was characterized by the presence of the centrilobular and focal necrosis with numerological reduction in the hepatic cells and vacuolization of the cytoplasm. Similar hepatopathological changes were also reported by Sauer et al., (1997), Mitsumori et al., (1998) and Horiguchi et al., (2000). The cadmium hepatotoxicity was successfully dealt by application of antioxidant like vitamins Kudo et al., (1986) and Tziroginnis et al., (2004). These antioxidants attack the free cadmium ions. These free ions are removed by these antioxidants forming a complex resulting in detoxification of tissue.

Furthermore, supplementation with dietary nutrients either individual or in combination exerted a protective effect in Cd intoxicated rats which was evident from the morphometric data (Table 34-36). Probably, such nephroprotective effects against
the development of histopathological changes might be attributed partly to the antitoxic
action of nutrients which promote antioxidant activity and scavenge the free radicals
from kidney. This reversal of the morphological and histological changes by dietary
supplementation is consistent with the results of many investigators (Yiin et al., 1999a;
Newairy et al., 2007). Moreover, *Spirulina platensis* significantly attenuated these
structural changes induced by Cd intoxication, which has also been documented by
Kuhad et al., (2009).

In agreement with the previous results, current study revealed that the levels of
LPO were significantly higher in Cd group than control animals in the tissue. Reversal of
these changes by dietary nutrients is consistent with the results of many investigators
(Caylak et al., 2007), who recorded that the tissues treated with NAC shows inhibition of
lipid peroxides in rats. Similarly, supplementation of Se (Yiin et al., 2000), Vit-E
(Hassan et al., 2007), Zn (Jihen et al., 2008), methionine (Patra et al., 2004) and
melatonin (Konar et al., 2007) showed protection against Cd-induced toxicity.

In the present study, methionine, NAC, Vit-B₁, melatonin and Zn were effective
detoxicant than the other dietary nutrients. This was also reported by Kim et al., (1998)
and Jihen et al., (2008) that melatonin and zinc functions as efficient detoxicants. The
combination of dietary nutrients accelerated the proliferation of hepatic cells than the
control rats. It was suggested that combination treatment was most effective for
histopathological hepatic and renal alterations (Plate 60 & 94).

Vitamin E has been shown to act as an antioxidant in cells, interrupting the
propagation of LPO in the plasma membrane and thus preserving membrane integrity.
Cadmium induced alterations in the biochemistry of liver and kidney could be reduced
by the simultaneous administration of α-tocopherol acetate. Vit-E is an important
antioxidant in biological systems. It inhibits peroxidation of membrane lipids by
scavenging lipid peroxyl radicals and is converted into a tocopheroxyl radical as a
consequence (Valko et al., 2006). The present results showed that treatment with Vit-E
alone or accompanied with cadmium chloride caused a highly significant decrease in the
levels of MDA and an increase in SOD, GPx, ALP activities and total protein level as
compared with cadmium chloride treated group and control group respectively (Table
24-31). The presence of Vit-E with cadmium alleviated its harmful effect on all the
above measured parameters. The observed levels of these parameters were close the normal values of the control. These results are in good accordance with those obtained by Valko et al. 2006 and El-Demerdash et al., 2004 who found that Vit-E maintained the levels of antioxidants, membrane-bound enzymes and the activities of antioxidant enzymes near normal levels, thus emphasizing its effect as antioxidants.

Zinc has been shown to have antioxidant effect, which was reviewed by Bary and Bettger (1990). Beside some proposed mechanisms for the antioxidant function of zinc two mechanisms have been elucidated (i) the protection of sulfhydryl groups against oxidation and (ii) prevention of ROS (HO and O2) production by transition metals. Simultaneous dietary nutrients supplementation with zinc + methionine/thiamine was found to effectively reverse, inhibition of lead sensitive zinc dependent enzyme δ-aminolevulinic acid dehydratase (ALAD) activity in the blood (Flora et al., 1995).

Cadmium has inhibitory effects on a number of zinc containing enzymes. Cadmium is also known to replace zinc in metallothionein. Therefore, cadmium toxicity could be treated by zinc supplementation, suggesting antagonism between the two metals. Our results indicated that one of the mechanisms involved in the protective role of Zn against Cd-induced toxicity is connected with inhibition of Cd-induced ROS formation. The role of Zn in protecting biological structures from free radical damage may be due to by maintaining an adequate level of metallothionein, which is also a free radical scavenger (Santon et al., 2003).

NAC was co-administered at an early phase of chronic Cd exposure in order to boost the antioxidant system. NAC can serve as a cysteine donor for GSH synthesis. GSH is an antioxidant and can also form complexes with Cd to alter Cd distribution and excretion (Rana and Verma, 1996). However, NAC did not cause further elevation in GSH levels in Cd administered rats. However, the production was shown leading to the scavenging free radicals and protection against oxidative damage. Furthermore, NAC protected against ultraviolet-B radiation-induced immune suppression in mice and against cold ischemic are perfusion injury in rat liver, even though GSH levels were depleted by pretreatment with BSO (Buthionine sulfoximine) (Nagasaki et al., 1998; Steenvoorden et al., 1998).
Yiin et al., (2000) noticed that selenium reduced the accumulation of Cd in the liver and kidney which may lead to a decrease in hydroperoxide level. The ameliorating effect of Se on biochemical parameters (ALT, AST, GGT, ALP and bilirubin) might be either due to interaction of Se with Cd, forming biologically inactive cadmium selenide complexes (Whanger et al., 1980) or due to decreased lipid peroxidation, antioxidant property and scavenging free radicals in liver and kidney (Miller et al., 2007). This may also increase the hepatic glutathione content. Yuan and Tang (1999) reported that selenium is one of the necessary trace elements in the body which has the ability to counteract free radicals. Selenium has antioxidant properties because of its presence in the active center of glutathione peroxidase.

Methionine is the preferred substrate for glutathione production by hepatocytes and acts as a precursor for glutathione production in the liver (Reed et al., 1997). Lead exposed rats treated with 100 mg/kg body weight of methionine demonstrated a significant decline in lipid peroxide in the liver (Patra et al., 2001). Methionine has been shown to react with ROS to form methionine sulfoxide and to increase ROS scavenging by improving hepatic glutathione levels (Reed et al., 1997). Methionine supplementation leads to increase in thiol molecule groups, of sulfur based protein that act as antioxidants to prevent peroxidation in the liver and kidneys of lead or alcohol.

Melatonin is an established free radical scavenger and was observed by several researchers recently (Konar et al., 2007). It was reported that melatonin had an antioxidant effect, whereby, it quenched hydroxyl radicals, superoxide anion radicals, and singlet oxygen and peroxide radicals and that it was also effective as a SOD and GPx stimulator (Longoni et al., 1998; Tan et al., 2002; Chwelatiuk et al., 2006). Noda et al. (1999) stated that when rats were administered 1 mg/kg subcutaneous cadmium and 10 mg/kg intraperitoneal melatonin for 15 days, there was a decrease in hepatic damage caused by cadmium. Karbownik et al. (2001) also reported that melatonin restored the lipid peroxidation caused by cadmium in hamsters. The results of our study demonstrated that individual and combined melatonin administration significantly prevented the oxidative stress brought about by cadmium (p<0.05). Our findings are consistent with those of the aforementioned researchers. The effect of melatonin is
attributable both to its being a SOD and GPx stimulator and its radical scavenging effect (Tsia et al., 2003).

Cysteine, cystine and methionine are the principle sources of sulfur in the diet of man. Cysteine is present in the body partly in the form of cystine, which is formed by oxidation of the thiol (-SH) groups of two cysteine molecules to form a disulfide bridge. Methionine is important in its function as a donor of methyl groups in various transmethylation reactions including synthesis of choline and creatine (Mangoni et al., 2002).

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 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 (Amin et al., 2008). *Spirulina* is rich in beta-carotene and the bioavailability is as good as the pure beta-carotene 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. *Spirulina* has been known to restore the level of antioxidant enzymes and oxidative stress markers against cyclophosphamide and mitomycin C and cisplatin and urethane (Premkumar et al., 2004) in mice. The protective effect of *Spirulina* against Cd induced oxidative stress in this study may also be attributed to its antioxidant and chelating effects (Jeyaprakash et al., 2005). It has been reported that *Spirulina* possesses strong antioxidant and free radicals scavenging properties (Mazo et al., 2004; Wu et al., 2005).

In conclusion, this study demonstrated that oral supplementation of individual dietary nutrients or their combination protect against Cd induced lipid peroxidation and ameliorated the negative effects of Cd on antioxidant status with lowering the Cd levels in tissues, thus act by mechanisms different from therapeutic approaches. However, this
antioxidative capacity of nutrients became most effective when administered in combination (Met+Zn+Vit-B1+NAC).

*Spirulina* is being considered as one of the nutritionally enriched naturally occurring food consisting of protein, minerals, vitamins, amino acids, essential, and essential fatty acids and rich source of antioxidants which has been proved to combat oxidant damage and protected against Cd induced nephro-, hepato- and haematoxicity and this effect has been attributed to its antioxidative and antiperoxidative properties.