Journal of Medical Sciences

ISSN 1682-4474

science alert

ANSInet
an open access publisher
http://ansinet.com

Research Paper

J. Med. Sci., 6 (4): 615-620
July-August, 2006

Pretreatment of Rats with α-tocopherol Alter Liver and Kidney Protein, Alkaline Phosphatase Activity and Phospholipid Profile after 24 Hour Intoxication with Cadmium

G.E. Eriyamremu, M.A. Adaikpoh and F.O. Obi

Cell membrane composition and fluidity are altered in diseases and previous reports suggest that membrane lipid is altered in heavy metal toxicity. This study was carried out to assess the effect of cadmium alone and its combination with different doses (75, 150 and 750 mg) of α-tocophenylacetate on the phospholipid profile and alkaline phosphatase activity in the kidney and liver of rats. The results obtained show that in these tissues, cadmium significantly ($p<0.05$) increased the levels of protein compared with the control, in the liver it significantly raised total lipid levels and decreased it in the kidney. Vitamin E in the form of α-tocophenylacetate reversed these observations. Cadmium decreased alkaline phosphatase activity significantly ($p<0.05$) in both tissues; a trend that was counteracted by α-tocophenylacetate supplementation in a dose dependent pattern. In both the liver and kidney, cadmium treatment reduced the levels of phosphatidylcholine (PC), phosphatidylethanolamine (PE) and increased sphingomyelin (SGM). It raised the PC/PE and SGM/PC ratios, index of membrane fluidity. Vitamin E supplementation significantly reduced the SGM/PC ratios in a manner that appears to be dose related. In the liver the effect of the vitamin on the SGM/PC ratio range from about 400 to 300 to 800% in the 75, 150 and 750 mg supplemented groups, respectively. A similar trend was also observed in the kidney. We hypothesize that decreased membrane fluidity occasioned by increase in SGM/PC ratio occur in early cadmium toxicity and that vitamin E cushions the effect of cadmium by decreasing SGM/PC ratio.

Key words: α-tocophenylacetate, cadmium, phospholipids, alkaline phosphatase

JMS (ISSN 1682-4474) is an International, peer-reviewed scientific journal that publishes original article in experimental & clinical medicine and related disciplines such as molecular biology, biochemistry, genetics, biophysics, bio-and medical technology. JMS is issued six times per year on paper and in electronic format.

For further information about this article or if you need reprints, please contact:

G.E. Eriyamremu
Department of Biochemistry,
University of Benin, P.M.B. 1154,
Benin City, Nigeria

ANSInet
Asian Network for Scientific Information

Department of Biochemistry, University of Benin, P.M.B. 1154,
Benin City, Nigeria

INTRODUCTION

Exposure to toxic metals has become an increasingly recognized source of illness worldwide. Cadmium is ubiquitous in the environment and a known industrial pollutant. Exposure through food, water, as well as occupational sources has for decades been known to cause a variety of adverse effects, among which are hepato-renal toxicity (Habeebu *et al*., 2000), Lung and bone (Zacharias *et al*., 2001; Brzoska *et al*., 2000) diseases. Mechanisms of cadmium toxicity remain incompletely understood, but it is becoming accepted that cadmium causes tissue damage by membrane lipid peroxidation due to its ability to generate free radicals and with concomitant impairment of the body's natural antioxidant systems (Baghi *et al*., 1996; Shaikh *et al*., 1999; Patra *et al*., 1999).

Oxidative stress is a well-known factor promoting cell damage and apoptosis (Antunes *et al*., 2001; Li *et al*., 1997) and has been implicated in the pathogenesis of a variety of diseases (Bennett, 2001; Kluck *et al*., 1997). Apoptosis cause biochemical changes such as DNA fragmentation, caspase-3 activation and accumulation of phosphatidylserines in the outer leaf of plasma membranes (Hong *et al*., 2004). Though the role of individual phospholipids on apoptosis is not clear, variations in membrane phospholipids can produce changes in cellular membrane fluidity and/or permeability (Nelson *et al*., 1991; Dorrance *et al*., 2001). Also some enzymes which are known to carry out important regulatory function are affected by membrane phospholipids (Epand and Lester, 1990; Gavrilova *et al*., 1993). So oxidation of phospholipids may not only damage the membrane but may alter phospholipid type and thus membrane fluidity.

Vitamin E, a dietary antioxidant, is presumed to be incorporated into the lipid bilayer of biological membranes to an extent proportional to the amount of polyunsaturated fatty acids or phospholipids in the membrane (Sekharam *et al*., 1990). Because of its hydrophobic nature, vitamin E is readily accommodated within the hydrophilic lipid region of the membrane and lipoprotein where its ability to quench free radicals become readily useful (Traber and Sies, 1996). Being an important antioxidant, Vitamin E may interfere with cadmium toxicity by preventing auto-oxidation of membranes. Despite the several evidences of cadmium induced compromise of cell membranes, the number of studies concerning its effects on cell membrane phospholipid profile is limited. The aim of this study is to determine the effect of cadmium alone and its combination with vitamin E on the phospholipid profile of the liver and kidney in early cadmium toxicity.

MATERIALS AND METHODS

Twenty five male rats (Wistar strain; 130-170 g), purchased from the laboratory animal unit of the Lagos University Teaching Hospital, Lagos, Nigeria were used for this study. They were maintained on normal rat chow (Pfizer Feeds Nigeria Ltd.) and water *ad libitum* throughout the period of study. The animals were acclimatized to our laboratory conditions for a period of 2 weeks and thereafter, were weighed and randomly divided into 5 groups of 5 rats each such that the weight difference between the groups was about 0.3 g. They were weighed once a week and the feed intake and faecal output measured daily. Group A (control; -Vit-Cd) was not exposed to either cadmium or the diet supplemented with α-tocophenylacetate while Group B (-Vit+Cd) rats received cadmium alone (3 mg kg^{-1} body wt.). Groups C (+V75+Cd); D (+V150+Cd) and E (+V750+Cd) received 75 mg kg^{-1} body wt., 150 mg kg^{-1}body wt. and 750 mg kg^{-1} body wt. α-tocophenylacetate, respectively for six weeks before being administered with cadmium (3 mg kg^{-1} body wt.) subcutaneously. Twenty-four hours after cadmium administration the animals were sacrificed after been anaesthetized with chloroform. While under anesthesia, blood was obtained via heart puncture, thereafter the animals were sacrificed and the liver and kidney were excised. The tissues were then washed in normal saline and the biochemical assays were immediately carried out. Were the tissue could not be immediately assayed; the tissues were stored frozen at -20°C until required for biochemical assays which was within 48 h. Animals were handled in accordance with the principles of laboratory animal care as contained in NIH guide for laboratory animal welfare.

Tissue (20%) homogenate of the kidney and liver in normal saline was prepared and used for the estimation of total protein and assay of enzyme activity.

Extraction of lipids and biochemical assays: Total lipids of a known weight of the tissues (1 g) were extracted by the modified method of Bligh and Dyer (1959) and the amount of the total lipid was determined by the method of Frings and Dunn (1970). The fractions of the phospholipids were separated using thin layer chromatography as described by Cuzner and Davidson (1967). Spots corresponding to specific phospholipids were recovered by scrapping and quantified. For this quantification the method of Fiske and Subarrow (1925) was employed. Recovered phospholipid fractions and reference standards (containing between 1 to 5 μg P/tube) were digested with perchloric acid. The digests were then incubated with molybdate and ascorbic acid solutions and the absorbance was read at 800 nm.

Alkaline phosphatase activity was assayed using kits from Randox laboratories (England) while total protein was determined by the method of Lowry *et al.* (1951).

Statistical analysis: Statistical analysis was performed using ANOVA. The Turkey-Kramer Multiple Comparison Test was used to determine differences between means. Values were considered significant at $p<0.05$.

RESULTS

Statistical analysis of the data did not reveal any significant ($p>0.05$) difference in weight gain, feed consumption and the feed efficiency after 24 h of cadmium administration in rats fed α-tocopherol (Table 1).

In the liver, evaluation of the data revealed that cadmium significantly ($p<0.05$) increase protein and total lipid level of rats which was however reduced by α-tocophenylacetate (Table 2). While, cadmium treatment significantly ($p<0.05$) raised the level of total lipid in the liver it reduced it in the kidney. This change in tissue total lipid was resisted in the rats whose diets were supplemented with α-tocophenylacetate.

In liver and kidney a significant ($p<0.05$) reduction in alkaline phosphatase activity occasioned by cadmium administration was reversed by α-tocophenylacetate (Table 3).

The study shows that cadmium intoxication caused a significant decrease in the liver PC/PE and SGM/PC ratios (Table 4). Supplementing the rat diets with α-tocophenylacetate increased the PC/PE and the SGM/PC ratios in apparently a dose related manner. In the liver the effect of the vitamin on the SGM/PC ratio range from about 400 to 300 to 800% in the 75, 150 and 750 mg supplemented groups, respectively.

In the kidney α-tocophenylacetate resisted the decrease in the PC and PE levels occasioned by cadmium and caused a significant increase in the SGM/PC ratio (Table 5). On the contrary cadmium administration reduced the PC/PE ratio and the vitamin increased the PC/PE ratio.

DISCUSSION

This study has provided data on the effects of vitamin E (an antioxidant vitamin) pretreatment on changes that occur in phospholipid profile and alkaline phosphatase activity associated with tissue damage caused by acute sub-lethal dose of cadmium in rats.

The data presented in Table 1 are in consonance with an earlier report by Ima-Nirwana *et al.* (1998). Though lipids are known to improve palatability of foods which normally affects food intake, this vitamin may not affect palatability and did not affect food consumption though it affected feed efficiency. Evidently the rats were in a comparable physiological state before cadmium intoxication.

Increase in tissue total protein observed in the liver and kidney of rats exposed to cadmium alone (Table 2) may in part be due to the ability of this toxicant to induce

Table 1: Effects of α-tocopherol (vitamin E) pretreatment on food intake, weight gain and feed efficiency in 24 h cadmium intoxication

	Group				
Parameter	(1) -V-Cd	(2) -V+Cd	(3) +V75+Cd	(4) +V150+Cd	(5) +V750+Cd
Weight gain (g)	1.3±0.1	1.2±0.2	1.1±0.2	1.1±0.1	1.4±0.2
Feed consumption (g)	14.1±2.4	14.1±2.2	13.4±2.2	14.6±2.3	13.0±2.3
Feed efficiency (Wt. gain/feed intake)	0.092	0.085	0.082	0.075	0.108

Values are given as mean±SEM. Means of the same row did not differ significantly ($p>0.05$). V-Cd = group was not given vitamin or cadmium and acted as the sham control. -V+Cd = group was not given vitamin but treated with cadmium and it acted as the test control. +V75+Cd = group was given 75 mg vitamin E and administered cadmium. +V150+Cd = group was given 150 mg vitamin E and administered cadmium. +V750+Cd = group was given 750 mg vitamin E and administered cadmium

Table 2: Effects of α-tocopherol (vitamin E) pretreatment on kidney and liver protein and total lipid levels in 24 h cadmium intoxication

	Group				
Parameter	(1) -V-Cd	(2) -V+Cd	(3) +V75+Cd	(4) +V150 +Cd	(5) +V750 + Cd
Protein					
Kidney	9.2±1.2^{a}	14.0±2.2^{b}	12.4±2.1^{ab}	11.5±1.1^{a}	10.2±2.2^{a}
Liver	10.6±1.5^{a}	20.4±2.4^{b}	15.8±1.9^{c}	12.6±1.3^{ac}	11.1±1.9^{a}
Total lipid					
Kidney	5.4±0.1^{a}	3.0±0.4^{b}	5.8±0.2^{a}	5.7±0.2^{a}	6.0±0.1^{a}
Liver	5.0±0.6^{a}	8.0±0.2^{b}	6.0±0.8^{a}	7.0±0.9^{ab}	5.0±0.1^{a}

Values are given as mean±SEM. Means of the same row did not differ significantly ($p>0.05$). -V-Cd = group was not given vitamin or cadmium and acted as the sham control. -V + Cd = group was not given vitamin but treated with cadmium and it acted as the test control. +V75 + Cd = group was given 75 mg vitamin E and administered cadmium. +V150 + Cd = group was given 150 mg vitamin E and administered cadmium. +V750 +Cd = group was given 750 mg vitamin E and administered cadmium

Table 3: Effect of α-tocopherol (vitamin E) on kidney and liver alkaline phosphatase in 24 h cadmium intoxication

	Group				
Tissue	(1) -Vit-Cd	(2) -Vit + Cd	(3) +V75+Cd	(4) +V150+Cd	(5) +V750+Cd
Kidney	55.7±5.0^{a}	13.90±1.9^{b}	28.3±3.0^{c}	39.2±2.0^{d}	46.0±3.0^{e}
Liver	40.1±3.0^{a}	10.0±2.0^{b}	18.1±2.1^{c}	20.5±2.1^{cd}	26.1±3.2^{d}

Values are given as mean±SEM. Means of the same row followed by different letters differ significantly ($p<0.05$). -V -Cd = group was not given vitamin or cadmium and acted as the sham control. -V+Cd = group was not given vitamin but treated with cadmium and it acted as the test control. +V75+Cd = group was given 75 mg vitamin E and administered cadmium. +V150+Cd = group was given 150 mg vitamin E and administered cadmium. +V750+Cd = group was given 750 mg vitamin E and administered cadmium

Table 4: Effects of α-tocopherol (vitamin E) pretreatment on liver phospholipid profile in 24 h cadmium intoxication

	Group				
Phospholipid	(1) -Vit -Cd	(2) -Vit+Cd	(3) +V75+Cd	(4) +V150+Cd	(5) +V750+Cd
PC	0.8±0.1^{a}	0.7±0.1^{ab}	0.5±0.1^{b}	0.5±0.10^{b}	1.0±0.1^{a}
PE	7.2±1.1^{a}	2.5±0.4^{b}	2.5±0.1^{b}	1.5±0.01^{c}	8.0±0.3^{a}
SGM	9.2±1.1^{a}	11.8±0.9^{b}	2.3±0.1^{c}	2.9±0.01^{c}	2.9±0.1^{c}
PC/PE ratio	0.1	0.3	0.2	0.3	0.3
SGM/PC ratio	11.5	16.9	4.6	5.8	2.9

Values are given as mean±SEM.Value are multiplied by 10^{-3}. Means of the same row followed by different letters differ significantly ($p<0.05$). -V-Cd = group was not given vitamin or cadmium and acted as the sham control. -+Cd = group was not given vitamin but treated with cadmium and it acted as the test control. +V75+Cd = group was given 75 mg vitamin E and administered cadmium. +V150+Cd = group was given 150 mg vitamin E and administered cadmium. +V750+Cd = group was given 750 mg vitamin E and administered cadmium. PC = phosphatidylcholine, PE = phosphatidylethanolamine, SGM = sphingomyelin

Table 5: Effects of α-tocopherol (vitamin E) pretreatment on kidney phospholipid profile in 24 h cadmium intoxication

	Group				
Phospholipid	(1) -V-Cd	(2) -V+Cd	(3) +V75+Cd	(4) +V150+Cd	(5) +V750+Cd
PC	1.1±0.2^{a}	0.5±0.1^{b}	0.5±0.1^{b}	1.3±0.3^{a}	1.2±0.2^{a}
PE	1.2±0.2^{a}	0.6±0.1^{b}	0.5±0.1^{b}	1.3±0.3^{a}	1.3±0.3^{a}
SGM	0.9±0.1^{a}	1.1±0.1^{a}	1.0±0.1^{a}	0.9±0.1^{a}	0.9±0.1^{a}
PC/PE ratio	0.9	0.8	1.0	1.0	0.9
SGM/PC ratio	0.8	2.2	2.0	0.7	0.8

Values are given as mean±SEM. The values were multiplied by 10. Means of the same row followed by different letters differ significantly ($p<0.05$). -V-Cd = group was not given vitamin or cadmium and acted as the sham control. -V+Cd = group was not given vitamin but treated with cadmium and it acted as the t-est control. +V75+Cd = group was given 75 mg vitamin E and administered cadmium. +V150+Cd = group was given 150 mg vitamin E and administered cadmium. +V750+Cd = group was given 750 mg vitamin E and administered cadmium. PC = phosphatidylcholine, PE = phosphatidylethanolamine, SGM = sphingomyelin

metallothionein synthesis (Klaassen and Liu, 1998). These two organs are known to accumulate cadmium (WHO, 1992; Brzoska *et al*., 2000) and appear to be inherently endowed with the gene(s) for metallothionein production. It is possible and remains to be demonstrated that cadmium not only activates metallothionein gene(s) but also cross-activates neighbouring gene(s). This situation may be a likely explanation for the increased protein levels (Table 2). In the kidney and liver, cadmium-induced proteins increase was generally counteracted by α-tocophenylacetate supplementation (Table 2). This trend is not in harmony with the pattern reported for lead intoxication in rats, in which vitamin E caused increases in the activities of Na^{+}/K^{+}; Ca^{2+} and Mg^{2+}ATPases (all proteins) (Upasani and Balaraman, 2001). Evidently α-tocophenylacetate can enhance or impair protein synthesis and/or enzyme activity depending on the nature of the heavy metal used for toxicity studies.

Alkaline phosphatase is quite ubiquitous in living tissues. It is present in the liver, bone, kidney, intestine, and placenta as well as in the breast during lactation (Nakanura *et al*., 1988). Zinc is a constituent of this enzyme (Moss and Handerson, 1999). The cadmium-induced inhibition of many metalloenzymes is reported to be due to the displacement of metals from the active site of the enzymes (WHO, 1992; Brzoska *et al*., 2000). Present findings in this study (Table 3) show a decrease in alkaline phosphatase activity in all cadmium-exposed rats. Similar results have been published by Garcia and Corredor (2004). However, it is interesting that this impairment in alkaline phosphatase activity was ameliorated by α-tocophenylacetate supplementation; an effect which appears to be dose dependent (Table 3).

The observation of the effect of cadmium and vitamin E on the phospholipid content of liver and kidney (Table 4 and 5) differ from those of Upsani and Balaraman (2001) which reported increased phospholipid levels in rat heart, kidney, liver and lung by lead. It is pertinent though to stress that we used acute low dose of cadmium (3 mg kg/body weight) which was administered subcutaneously and the animals were sacrificed after 24 h in this study; while Upsani and Balaraman (2001) used chronic low dose of lead (100 ppm) administered orally with a longer term exposure. These factors influence *in vivo* biological action of toxicants in a remarkably variable and idiosyncratic fashion.

Increase in SGM level observed in the liver and kidney (Table 4 and 5) agrees with those that had been reported in aging, during diseases development and in malignancy (Barenholz and Thompson, 1980). Thus the elevation of SGM in rats treated with cadmium may indicate the development of a disease. Sphingomyelin accounts for approximately 10 to 15% of total plasma membranes phospholipid content. It resides predominantly in the outer leaflet of the plasma membrane bilayer, forming tight hydrophobic interactions with cholesterol where it contributes to membrane fluidity (Lund-Katz *et al*., 1988; Grönberg and Slotte, 1990). An increase in the SGM of membranes is associated with a decrease in the microviscosity and fluidity of the membrane (Barenholz and Thompson, 1980; Dorrance *et al*., 2001) which leads to the decrease in the transport of non-electrolytes for which no specific transport system exists and affects enzyme action (Barenholz and Thompson, 1980; Sanderman, 1987). Thus the increase in tissue SGM induced by cadmium in this study would impair the entrance of some biomolecules which may be essential for the sustenance of tissue or organ. This may also have contributed to low alkaline phosphatase activity recorded in the cadmium treated rats. We speculate that this may be an important mechanism by which cadmium accelerate the development of diseases associated with its toxicity. The observed effect of vitamin E on SGM in this study implies that the vitamin may offer some protection to rats against disease development. Vitamin E may exert this effect by possibly affecting the activity of sphingomyelinase, which would reduce SGM. The hydrolysis of the lipid component of biological membranes might be expected to result in lysis, but earlier researchers have shown that the hydrolysis of 80% of SGM of human erythrocytes to ceramide and phosphorylcholine by sphingomyelinase which remain in the membrane do not lead to loss of membrane integrity and do not cause hemolysis (Barenholz and Thompson, 1980). So the decrease of SGM occasioned by α-tocophenylacetate may not result in membrane lysis and may indeed improve the chance of the tissue survival. Also, over the past several years, there have been increasing suggestions that activation of sphingomyelinase mediate potentially important signaling processes (Zhang and Kolesnick, 1994; Testi, 1996; Hannun, 1996). The possible effect of vitamin E on membrane sphingomyelinase activity in cadmium toxicity is worthy of further study to elucidate its effect on SGM of membranes.

We also used phosphatidylcholine (PC) and phosphatidylethanolamine (PE) ratio as indices of membrane fluidity and so establish whether or not cadmium alters this important membrane phenomenon. The use of this ratio to establish membrane fluidity is not new. Hirata and Axelrod (1980) used PC/PE ratio to assess membrane fluidity and transmembrane communication. It has also been reported that experimental incorporation of phosphatidylglycerol (PG) and dioleoylphophatidylcholine (DOPC) into isolated plasma membrane increased its fluidity (Gavrilova *et al*., 1993). Using the PC/PE ratio (Table 4 and 5), it was found that the toxicant caused decreased membrane fluidity in the kidney. Thus the use of both the PC/PE and SGM/PC ratios in this study show that decreased membrane fluidity is a pre-requisite in cadmium toxicity.

In conclusion, this study shows that an early event in cadmium toxicity is a decrease in membrane fluidity and may affect some membrane enzyme function such as alkaline phosphatase and that α-tocophenylacetate supplementation improve membrane fluidity thus suggesting a protective effect of the vitamin against cadmium toxicity. Confirming this effect of cadmium and vitamin E on membrane fluidity in long term studies as well as their effect on sphingomyelinase is worth further study.

REFERENCES

Antunes, F., E. Cadenas and U.T. Brunk, 2001. Apoptosis induced by exposure to a low steady-state concentration of H_2O_2 is a consequence of lysosomal rupture. Biochem. J., 365: 549-555.

Baghi, D., M. Bagchi, E.A. Hassoun and S.J. Stohs, 1996. Cadmium induced excretion of urinary lipid metabolites, DNA damage, glutathione depletion and hepatic lipid peroxidation in Spragne-Dawley rats. Biol. Trace Elem. Res., 52: 143-154.

Barenholz, Y. and T.E. Thompson, 1980. Sphingomyelins in bilayers and biological membranes. Biochim. Biophys. Acta, 604: 129-158.

Bennett, M.R., 2001 Reactive oxygen species and death: Oxidative DNA damage in atherosclerosis. Circ. Res., 88: 648-650.

Bligh, E.G. and W.J. Dyer, 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol., 37: 911-917.

Brzoska, M.M., J. Moniuszko-Jakoniuk, M. Jurezuk, M. Galazyn-Sidorezuk and J. Rogalska, 2000. Effect of short term ethanol administration on cadmium retention and bioelement metabolism in rats continuously exposed to cadmium. Alcohol and Alcoholism, 35: 439-444.

Cuzner, M.L. and A.N. Davidson, 1967. Quantitative thin layer chromatography of lipids. J. Chromatog., 27: 388-397.

Dorrance, A.M., D. Graham, R.C. Webb, R. Fraser and A. Dominiczak, 2001. Increased membrane sphingomyelin and arachidonic acid in stroke-prone spontaneously hypertensive rats. Am. J. Hypertens., 14: 1149-1153.

Epand, R.M. and D.S. Lester, 1990. The role of membrane biophysical properties in the regulation of protein kinase C activity. Trends Pharmac. Sci., 7: 317-320.

Fiske, C.H. and Y. Subarrow, 1925. The Colorimetric determination of phosphorus. J. Biol. Chem., 66: 375-400.

Frings, C.S. and R.T. Dunn, 1970 A colorimetric method for determination of total serum lipids based on the sulfophospho-vanillin reaction. Am. J. Clin. Pathol., 53: 89-91.

Garcia, T.A. and L. Corredor, 2004. Biochemical changes in the kidneys after perinatal intoxication with lead and./or cadmium and their antagonistic effects when coadministered. Ecotoxicol. Environ. Safety, 57: 184-189.

Gavrilova, N.J., M.S. Setchenska, T.T. Markovska, A.B. Momchilova-Pankova and K.S. Koumanov, 1993. Effect of membrane phospholipid composition and fluidity on rat liver plasma membrane tyrosine kinase activity. Intl. J. Biochem., 25: 1309-1312.

Grönberg, L. and J.P. Slotte, 1990. Cholesterol oxidase catalyzed oxidation of cholesterol in mixed lipid monolayers: Effects of surface pressure and phospholipid composition on catalytic activity. Biochemistry, 29: 3173-3178.

Habeebu, J.L., L. Naging and D.C. Klaassen, 2000. Biochemistry M.T. Null mice are more sensitive than wild mice to liver injury induced by repeated exposure to cadmium. Toxicol. Sci., 55: 223-232.

Hannun, Y.A., 1996. Functions of ceramide in coordinating cellular responses to stress. Science, 274: 1855-1859.

Hirata, F. and J. Axelrod, 1980. Phospholipid methylation and biological signal transduction. Science, 209: 1082-1090.

Hong, H., L. Hong, W. Huizhen, W. Jingxiong, Z. Yiqiang and W. Zhiguo, 2004. Progressive apoptotic cell death triggered by transient oxidative insult in H9c2 rat ventricular cells: A novel pattern of apoptosis and the mechanisms. Am. J. Physiol., 286: H2169-H2182.

Ima-Nirwana, S., M. Norazlina, A.B.D. Gapor, M.D. Top and B.A.K. Khalid, 1998. Vitamin E deficiency impairs weight gain in normal and ovariectomised growing female rats. Med. J. Islamic Acad. Sci., Vol. 11.

Klaassen, C.D. and J. Liu, 1998. Induction of metallothionein as an adaptive mechanism affecting the magnitude and progression of toxicological injury, Environ. Health perspect., 106: 1-8.

Kluck, R.M., E. Bossy-Wetzel, D.R. Green and D.D. Newmeyer, 1997. The release of cytochrome *c* from mitochondria: A primary site for Bcl-2 regulation of apoptosis. Science, 275: 1132-1136.

Li, P., R. Dietz and R. von Harsdorf, 1997. Differential effect of hydrogen peroxide and superoxide anion on apoptosis and proliferation of vascular smooth muscle cells. Circulation, 96: 3602-3609.

Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall, 1951. Protein measurement with the folin-phenol reagent. J. Biol. Chem., 193: 265-275.

Lund-Katz, S., H.M. Laboda, L.R. McLean and M.C. Phillips, 1988. Influence of molecular packing and phospholipid type on rates of cholesterol exchange. Biochemistry, 27: 3416-3423.

Moss, D.W. and A.R. Handerson, 1999. Clinical Enzymology. In: Burtis, C.A. and E.R. Ashwood (Eds.), Tiets Texbook of Clinical Chemistry (3rd Edn.), Philadelphia, Saunders WB., pp: 617-721.

Nakanura, I., K. Nakamura and RA. Stisson, 1988. Release of alkaline phosphatase from human osteosarcoma cells by phosphatidylinositol phospholipase C: Effect of Tunicamycin. Arch. Biochem. Biophys., 265: 190-196.

Nelson, J.M., P.G. Duane, K.L. Rice and D.E. Niewoehner, 1991. Cadmium ion induced alteration of phospholipid metabolism in endothelial cell. Am. J. Respr. Biol., 5: 328-336.

Patra, R.C., D. Swarup, S.K. Senapati, 1999. Effects of cadmium on lipid peroxides and superoxide dismutase in hepatic, renal and testicular tissue of rats, Vet. Hum. Toxicol., 41: 65-66.

Sanderman, H., 1987. Regulation of membrane enzymes by lipids. Biochem. Biophys. Acta, 515: 209-237.

Shaikh, Z.A., J.T. Vu and K. Zaman, 1999. Oxidative stress as a mechanism of chronic cadmium induced hepatotoxicity and renal toxicity and protection by antioxidants. Toxicol. Appl. Pharmacol., 154: 256-263.

Sekharam, K.M., J.M. Patel and E.R. Block, 1990. Effect of polyunsaturated fatty acids and phospholipids on (sup 3 H)-vitamin E incorporation into pulmonary artery endothelial cell membranes. J. Cell. Physiol., 145: 555-563.

Testi, R., 1996. Sphingomyelin breakdown and cell fate. Trends Biochem. Sci., 21: 468-471.

Traber, M.G. and H. Sies, 1996. Vitamin E in humans: Demand and delivery. Ann. Rev. Nutr., 16: 321-347.

Upasani, C.D. and R. Balaraman, 2001 Effect of vitamin E, vitamin C and spirulina on the levels of membrane bound enzymes and lipids in some organs of rats exposed to lead. Indian J. Pharmacol., 33: 185-191.

WHO, 1992. Environmental Health Criteria, 134, Cadmium, World Health Organization, Geneva, pp: 111-112.

Zacharias, B., H.J. Lantzch and W. Drochner, 2001. The influence of dietary microbial phytase and calcium on the accumulation of cadmium in different organs of pigs. J. Trace Elem. Med. Biol., 15: 109-114.

Zhang, Y. and R. Kolesnick, 1994. Signaling through the sphingomyelin pathway. Endocrinology, 136: 4157-4160.