Australian Journal of Basic and Applied Sciences, 5(5): 1178-1183, 2011
ISSN 1991-8178

Roles of N-acetylcysteine, Methionine, Vitamin C and Vitamin E as Antioxidants Against Lead Toxicity in Rats

Madiha, M. Abdel-Kader; Abeer, A. Afify; Amany, M. Hegazy

Food Science & Nutrition Dept., National Research Center, Dokki, Cairo, Egypt

Abstract: Humans are exposed to a number of toxic elements in the environment. Lead, widely used in industry, is a great environmental health problem of both humans and animals. The present study was designed to investigate the protective effect of sulpher-containing antioxidants (N-acetylcysteine (NAC) and methionine (Meth.) as well as vitamin C and vitamin E) against the lead toxicity. Experiments were carried out on rats given lead acetate in their drinking water for 4 weeks (1000 ppm) with or without relatively high doses of the mentioned antioxidants. Several biochemical parameters representing different organs function were followed. The results showed significant decrease in hemoglobin (Hb) in the lead (Pb) group. Whereas malondialdehyde (MDA) levels were significantly higher compared to the normal control group. Pb- NAC and Pb-Meth. groups were found to be very effective in increasing Hb levels when compared to those in other Pb- antioxidant groups. Marked reduction was observed in MDA concentrations in Pb- antioxidant groups when compared to Pb- group. Meanwhile the vitamin C treatment group had non significantly decrease. There was a decrease below Pb- control group values in erythrocyte superoxide dismutase (SOD) and glutathione peroxidases (GSH-Px) levels in the Pb- antioxidant groups. The plasma Vit. C levels in the Pb- antioxidant rat groups were lower than the normal control rats, but not significantly. A significant increase could be noted in Vit.A contents in plasma from the Pb- NAC and Pb- Meth. supplemented groups compared to Pb- group. Plasma Vit.E. contents were markedly higher in rats given Pb- Vit. E or Pb in conjunction with NAC when compared to Pb- control group. A noticeable increase in the activity of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) as liver functions occurred due to lead acetate in the drinking water. In conclusion, the results in this study indicate that the oxidative stress induced by lead is reduced by the all investigated antioxidants. Also the hazards due to ingestion of lead-acetate can be partially corrected if the intake from sulphur-containing compounds and vitamins is increased.

Key words: Lead toxicity, oxidative stress, N-acetylcysteine, methionine, vitamin C, vitamin E.

INTRODUCTION

Lead is one of the most abundant heavy metals on earth. It has been widely used throughout human history, posing a serious health problem to susceptible populations, such as children or occupationally exposed people (Jung-Hun *et al*., 2007). It commonly used in industrialized countries, adversely affects human and animal physiological, biochemical and behavioral functions such as kidney dysfunction and impairment of liver function. Lead intoxication is a complex disorder that affects several cells and organs, including functional and structural alteration of erythrocytes (Koller *et al*., 2004; Calderon- Salinas *et al*., 1999). One of the most prominent effects of lead is exerted on the biosynthesis of hem, the prosthetic group present hemoglobin, cytochromes, catalases and peroxidases (Needleman, 2004). Some researchers have investigated the benefit of antioxidants in preventing lead toxicity, the mechanisms of antioxidant nutrients being effective via rebalancing the impaired pro-oxidant / antioxidant ratio are not completely clear. Antioxidant nutrients including, vitamin E, vitamin C, vitamin B6, B-carotene, zinc, and selenium (Kedziora- Kornatowska *et al*.,2003; Elayat and Bakheet, 2010). The strong scientific interest in the role of antioxidants has expanded the focus of research from reducing the oxidative stress of lead exposure to improving the pro-oxidant / antioxidant balance of cells. Thiol-containing compounds bind lead at their-SH (thio) groups and have antioxidant features. Therefore, thiol-containing antioxidants may be useful as a component of an effective treatment for lead poisoning. Several previous studies have suggested that lead- induced oxidative damage in red blood cells may result from direct

Corresponding Author: Madiha, M. Abdel-Kader; Food Science & Nutrition Dept., National Research Center, Dokki, Cairo, Egypt
E-mail: Madeha65@yahoo.com

interaction of lead with their membranes, inducing lipid peroxidation (Sandhir *et al*., 1994; Caylak *et al*., 2008). N-Acetylcysteine (NAC) has antioxidant capacity to lead, including oxidative stress via stimulating glutathione synthesis, thereby maintaining inter acellular glutathione levels and scavenging reactive oxygen species (Ereal *et al*., 1996). In addition, NAC also has some chelating action on lead (Aruoma *et al*., 1989). Methionine acts a precursor amino acid for glutathione which protects the cells from oxidative damage and plays vital role in detoxification (Reed, 1990). In addition, methionine has been shown to chelate lead and remove it from tissues (Patra *et al*., 2001). Both vitamin C and vitamin E decreased lipid peroxidation and augmented the activities of antioxidant enzymes studied in the kidneys and liver of rats. (levander *et al*., 1980; Ribarov *et al*., 1981; Kedziora- Kornatowska *et al*.,2003).

Little information is available in the literature regarding the antioxidant activity of thiol-containing amino acids (N-Acetylcysteine and methionine). Therefore, the present study was undertaken to investigate the antioxidant activity of these sulphur containing amino acids of rats received lead acetate in their drinking water for 4 weeks. Also the antioxidant activity of vitamin C and E in the same rats was studied.

MATERIALS AND METHODS

Animals:

Sprague-Dawley rats were procured from the Central Animal House, National Research Center. They were housed in stainless steel cages in a temperature-controlled room (20-22°C) with a 12 hours light and 12 hours dark exposure.

Experimental Design:

Thirty sex Sprague- Dawley rats with an average weight (170- 200 g) were divided into 6 groups of 6 rats each. All groups were given only standard rat feed and water during the 1st week (Table 1). After this period the animals were fed to the following diets: Group (1) basal diet +Sodium acetate (1000 ppm) in their drinking water, group (2) basal diet + lead acetate (1000 ppm) in their drinking water, group (3) basal diet + N- acetyl cysteine (800ppm), group (4) basal diet + methionine (100ppm), group (5) basal diet +vitamin C (500ppm), group (6) basal diet + vitamin E (20ppm).The experiment continued for 4 weeks.

Analytical Measurements:

Parameters measured were, blood hemoglobin determined according to the method of Leong *et al*(2003), malondialdehyde(MAD)was determined as described by Ohkawa *et al*(1979), super oxide dismutase(SOD) was estimated as described by Roth and Gilbert(1984), glutathione peroxidases (GPx) was determined according to the method of kraus and Ganther (1980), aspartate aminotransferase (AST) was determined by the method of Friedman *et al*.(1980), alanine aminotransferase (ALT) was estimated as described by International Federation of Clinical Chemistry (1980), Vit.C determined by the method of Jagota and Dani (1982), Vit. E was determined according to Desai and Machlin (1985), Vit.A was estimated as described by Neeld and Pearson (1963), uric acid content was determined according to the method described by Barham, D.and Trinder, P. (1972). creatinine was estimated by the method of Larsen (1972).

Statistics:

All values are reported as mean± standard error. One way ANOVA analysis was used to compare between groups with statistical significance set at $p<0.05$.

Results:

Animals given lead acetate in drinking water for 4 weeks had significantly lower Hb concentration when compared with the normal control and other Pb antioxidant groups (Table 2). Although NAC and methionine were found to be very effective in increasing Hb levels when compared to those in other lead groups. Vit.C and E supplements did not appear to change Hb concentration. Blood Hb levels of supplemented groups with vitamin C and vitamin E were higher than that of lead group, but lower than those of NAC and Meth. and normal control groups.

Table (2) shows the results of serum MDA concentration in all tested groups. The MDA level of lead group was significantly higher than the control group values. Significant reduction was observed in MDA concentrations in all lead- antioxidant groups when compared to lead group. No significant decrease of MDA level was noted, in Vit. C treatment group, when compared with the normal control group.

Table 1: Composition of experimental diets [a] (g. /100g.).

Ingredients	Amount(g)
Casein	14.0
Sucrose	10.0
Corn oil	4.0
Cellulose	5.0
Salt mixture (AIN-93)*	3.5
Vitamin mixture (AIN-93)*	1.0
Choline chloride	0.2
Starch	62.3

[a:] The control diet content according to AOAC, (2000).

* Salt mixture and vitamin mixture according to Reeves *et al*., 1993.

Table 2: Effect of lead on blood hemoglobin, serum MDA,whole blood GSH and Erythrocytes SOD.

	Parameters			
Groups	Hb (g/dl)	MDA (n mol/ml)	GSH-Px (m mol/L)	SOD (U /g Hb)
Normal control (without Pb)	15.9 ± 0.396 [a]	5.03 ± 0.554 [b]	0.667 ± 0.055 [a]	1121.8 ± 49.3 [c]
Positive control (with Pb)	12.8 ± 0.373 [b]	6.31 ± 0.377 [a]	0.619 ± 0.026 [ab]	2177.3 ± 173.1 [a]
Pb + NAC	15.8 ± 0.567 [a]	3.39 ± 0.332 [cd]	0.552 ± 0.025 [b]	1465.3 ± 100.1 [b]
Pb + Meth.	15.4 ± 0.488 [a]	3.06 ± 0.111 [d]	0.313 ± 0.011 [d]	1277.1 ± 99.8 [bc]
Pb +Vit. C	14.6 ± 0.351 [a]	4.26 ± 0.409 [b]	0.429 ±0.038 [c]	1499.7 ± 50.2 [b]
Pb +Vit. E	14.9 ± 0.229 [a]	3.79 ± 0.347 [cd]	0.613 ± 0.033 [ab]	1437.8 ± 64.1 [b]

All values represent mean ± S.E. for 6 samples.

The activity of antioxidant enzymes SOD and GSH-Px in RBCs are shown in the same table. Significant decreases in SOD activity of erythrocytes were recorded in lead- Meth. group as compared with lead control group, but these effects were not significantly different than the other lead- antioxidant groups. Supplementing lead with the all tested antioxidants especially lead-Meth. and lead- Vit.C lowered the GSH- Px activities in RBCs as compared with the Pb- control group. Meanwhile the other investigated antioxidants did not.

The plasma vitamin C, A and E-levels are given in table (3).The plasma Vit. C levels in the Pb-antioxidant rat groups were lower than the normal control rats, but not significantly. Although no significant decrease was observed in the Pb- group and Pb-NAC as well as Pb- Meth. groups. A significant increase could be noted in Vit.A contents in plasma from the Pb- NAC and Pb- Meth. supplemented groups compared to Pb-group. Meanwhile Vit.C supplemented group decreased Vit.A levels than the normal control group and increased Vit.A concentration than the Pb- group. Plasma Vit.E. Contents were significantly higher in rats given Pb- Vit. E or Pb in conjunction with NAC when compared to Pb- control group. While the other antioxidant supplemented groups showed no significant differences between them when compared to normal control group.

Table (4) shows the change in the activity of AST and ALT of rats in the different groups. As shown in this table, a significant increase in these liver functions occurred due to lead acetate in the drinking water. The activity of AST and ALT in normal control group were 74.0±3.67 and 20.2±0.638 respectively increased to 93.6±7.06 and 24.6±2.89 respectively in Pb- control group. The all investigated antioxidants caused a significant decrease in the activity of these enzymes, when compared with those of Pb- group. Uric acid and creatinine levels of the same rats are given in the same table. Lead- group rats showed significantly increase in serum uric acid and creatinine values. Mean values of serum uric acid and creatinine showed non significant changes in the all Pb- antioxidant groups except Pb- Meth. and Pb- Vit. E groups respectively, when compared with the control groups. Significant decrease in uric acid concentration was recorded in Pb-Meth. group, compared to control groups.

Discussion:

Lead poisoning occurring in humans and animals either due to occupational or environmental exposure has become a great public problem lead alters virtually all biochemical processes and organ systems. Everyone is exposed to lead in air, household dust, food, drinking water and various consumer products. Acute and chronic lead exposure can seriously affect human health.It is a confirmed multi-target toxicant with effects on the gastrointestinal,haematopoietic,cardiovascular,nervous,immune,reproductive and excretory system (Daggett *et al*, 1998; Al-Neamy *et al*, 2001; D'Souza *et al*, 2007).

Oxidative stress also leads to lipid peroxidation in RBC membranes, autooxidation of hemoglobin and limited repair processes, leading to decrease survival (Rice-Evans and Baysal, 1987; Caylak *et al*., 2008).

The present study was designed to investigate oxidative stress parameters (Hb and MDA in serum, SOD and GSH-Px activities in erythrocyte hemolysate, and Vit.C,A and E in plasma as well as uric acid and creatinine in rats administered lead in conjunction with sulphur containing compounds (NAC and Methionine) as well as vitamins (C and E) as antioxidants.

Table 3: Effect of lead on serum vitamins C, A and E

	Parameters		
Groups	Vitamin C (mg/dl)	Vitamin A (µg/dl)	Vitamin E (mg/dl)
Normal control (without Pb)	1.11 ± 0.109 [a]	22.2 ± 0.539 [bc]	1.33 ± 0.111 [b]
Positive control (with Pb)	0.547 ± 0.034 [b]	17.56 ± 1.342 [d]	0.543 ± 0.051 [c]
Pb + NAC	0.789 ± 0.49 [ab]	28.9 ± 1.056 [a]	1.30 ± 0.218 [ab]
Pb + Meth.	0.797 ± 0.058 [ab]	29.1 ± 1.522 [a]	1.07 ± 0.193 [b]
Pb +Vit. C	1.01 ± 0.172 [a]	20.5 ± 1.612 [cd]	0.995 ± 0.083 [b]
Pb +Vit. E	0.996 ± 0.112 [a]	24.8 ± 1.769 [b]	1.61 ± 0.175 [a]

All values represent mean ± S.E. for 6 samples

Table 4: Effect of lead on some liver and kidney functions

	Parameters			
Groups	AST (U/ml)	ALT (U/ml)	Uric acid(mg/dl)	Creatinine(mg/dl)
Normal control (without Pb)	74.0 ± 3.67 [b]	20.2± 0.638 [ab]	14.4± 0.071[bc]	0.84± 0.01[c]
Positive control (with Pb)	93.6 ± 7.06 [a]	24.6 ± 2.89 [a]	16.5± 0.16[a]	1.10± 0.02[a]
Pb + NAC	63.5 ± 3.66 [b]	15.8 ± 0.717 [b]	14.1± 0.11[c]	0.86± 0.02[bc]
Pb + Meth.	71.7 ± 5.96 [b]	18.3 ±2.319 [b]	13.5± 0.17[d]	0.83± 0.05[c]
Pb +Vit. C	77.5 ± 4.21 [b]	17.5 ± 1.278 [b]	14.3± 0.4[c]	0.89± 0.02[bc]
Pb +Vit. E	68.3 ± 3.07 [b]	19.3 ± 0.833 [b]	14.5± 0.1 [bc]	0.90± 0.03 [bc]

All values represent mean ± S.E. for 6 samples

Most researches on lead toxicity in rats utilizes 4-5 weeks as lead exposure period in which they receive only water or therapeutic antioxidant compounds.In our study, lead and preventative antioxidant compounds were administered simultaneously in water to rats during the 4 weeks.

One of the most prominent effects of lead is exerted on the biosynthesis of heme, the prosthetic group present in hemoglobin (EL-Missiry, 2000; Lavicoli et.al., 2003; Needleman, 2004).Our results demonstrated that administration of lead acetate in the drinking water for 4 weeks induced a toxic effect on RBCs as indicated by a significant reduction in Hb concentrations, similar to other reports (Caylak *et al*., 2008; Elayat and Bakheet 2010).

Lead induced peroxidation results in the formation of aldehydic by- products such as MDA. In the present study, the increased MDA levels in serum of lead- exposed rats were significant confirming previous studies (Gurer *et al*., 1998, Kedziora- Kornatowska *et al*., 2003; Caylak *et al*., 2008).

In lead intoxication, lead induces generation of reactive oxygen species such as hydrogen peroxide (H_2O_2), superoxide ion ($O^{\cdot}_2{}^{-}$), singlet oxygen and hydroxyl radical ($HO^{\cdot}$). SOD and GSH-Px play an important role in protecting the cells against the toxic effects of $O^{\cdot}_2{}^{-}$and peroxides as well as H_2O_2, respectively, and may be upregulated in response to lead exposure (Caylak *et al*., 2008). Different effect of lead on the activities of SOD and GSH-Px was previously noticed. Sivaprasad *et al*., (2004) reported a drop of SOD and GSH-Px activities in lead exposed rats, while Soltaninejad *et al*., (2003) found higher activity of SOD. No significant effects of exposure to lead on the activities of SOD and GSH-Px were indicated (Caylak *et al*., 2008). In this work SOD has been observed elevated, whereas GSH-Px was decreased, when compared to normal control group.

We also determined the non enzymatic antioxidant status, e,g vitamins C, A and E in plasma of rats exposed to lead. In lead administrated rats; there is a decrease in these vitamin levels, similar to other previously reports (Attri *et al*., 2003; Caylak *et al*., 2008).

Lead can cause adverse effects to hepatic cells owing to its storage in the liver after lead exposure. Liver, being one of the major organs involved in the storage, biotransformation and detoxification of toxic substances, is of interest in heavy metal poisoning. The activities of liver enzymes namely AST and ALT of rats given lead acetate in their drinking water for 4 weeks were significantly increased in plasma .This is due to lyses of cells and the release of these enzymes into the circulation. Several studies have demonstrated changes in aminotransferase levels in rats exposed to Pb. The results of the present study are in accordance with the results reported by Sivaprasad *et al*. (2003), who reported an elevation in the levels of AST and ALT in rats exposed to 0.2% lead acetate in drinking water for a period of 5 weeks.

Levels of uric acid and creatinine in rats given lead acetate in their drinking water for 4 weeks were significantly increased in plasma. These results are in agreement with those reported by Ahrens (1993), who reported an increase of creatinine and uremia on the 30th day of the experiment in both male and female rates.

In conclusion, the hematological system is a major target of low-level lead exposure. The results obtained from this study showed that sulphur-containing antioxidants and vitamins supplementation proved to be beneficial to compensate hazards due to lead toxicity. The antioxidants preparation including the studied ones is necessary. The extrapolation of these findings to humans still needs investigation.

REFERENCES

Ahrens, F.A., 1993. Effects of lead on glucose metabolism, ion flux, and collagen synthesis in cerebral capillaries of calves. Am. J.Vet. Res., 54: 808-812.

Al-Neamy, F.R., A.M. Almehidi, R. Alwash, M.A. Pasha, A. Ibrahim and A. Bener, 2001. Occupational lead exposure and amino acid profiles and liver function tests in industrial workers. Int. J. Environ. Health res., 11: 181-188.

Aruoma, O.I., B. Halliwell, B.M. Hoey and J. Butler, 1989. The antioxidant action of n- acetylcysteine; its reaction with hydrogen peroxide, hydroxyl radical superoxide, and hypochlorous acid. Free Radical Biol. Med., 6: 593-7.

Association of Official Methods of Analysis International, 2000. "17th Ed., Gaithersburg, MD, Association of Analytical Chemistry international, U.S.A."

Attri, J., V. Dhawan, S. Mahmood, P. Pandhi, H.K. Parwana and R. Nath, 2003. Effect of vitamin C supplementation on oxidative DNA damage in an experimental model of lead- induced hypertension. Ann. Nutr. Metab, 47: 294-301.

Barham, D. and P. Trinder, 1972. Determination of uric acid with Uri case and peroxidases. Analyst, 97: 142-145.

Calderon-Salinas, J.V., M.A. Quintanar-Escorza, M.T. Gonzalez-Martinez and C.E. Hernandez-Luna, 1999. Lead and calcium transport in human erythrocyte. Human and Experimental Toxicology, 18: 327-332.

Caylak, E., M. Aytekin and I. Halifeoglu, 2008. Antioxidant effects of methionine, a-lipoic and N-acetylcysteine and homocysteine on lead- induced oxidative stress to erythrocytes in rats. Exp. Toxcol. Pathol., 60: 289-294.

D'Souza, Hs., M. Geraldine and T. Venkatesh, 2007. Evaluation, diagnosis and treatment of lead poisoning in a patient with occupational lead exposure: a case presentation. J. Occup. Med. Toxicol, 2: 7.

Daggett, D.A., T.D. Oberley, S.A. Nelson, L.S. Wright, S.E. Kornguth, F.L. Siegel, 1998. Effects of lead on rat kidney and liver: GST expression and oxidative stress. Toxicologyy, 128: 191-206.

Desai, I.D. and L.J. Machlin, 1985. Vitamin E. In: Methods of vitamin assay, Augustin, J., Klein, B. P., Beker, D., Venugopal, P. B. (Eds.) "Book" 4th Ed., A Wiley- Interscience publication, John Wiley and Sons, New York, pp: 255.

El- Missiry, M.A., 2000. Prophylactic effect of melatonin on lead- induced inhibition of heme biosynthesis and deterioration of antioxidant systems in male rats. J. Biochem. Toxicol., 14: 57-62.

Elayat, W. and M.S. Bakheet, 2010. Effect of chronic lead toxicity on liver and kidney functions. Journal of Medical Laboratory Science, 1(2): 29-36.

Ereal, N., P. Treeratphan, T.C. Hammond and R.H. Matthews, 1996. N-acetylcysteine protects Chinese hamster ovary (CHO) cells from lead- induced oxidative stress. Toxicology, 108: 57-64.

Friedman, R.B., R.E. Anderson, S.M. Entine and S.B. Hirshberg, 1980. Effects of diseases on clinical laboratory tests. Clin. Chem., 26(Suppl.4), ID-476D.

Gurer, H., H. Ozgunes, R. Neal, D.R. Spitz and N. Ercal, 1998. Antioxidant effects of N- acetylcysteine and succimer in red blood cells from lead- exposed rats. Toxicology, 128: 181-189.

International Federation of Clinical Chemistry, Scientific Committee, 1980. J. Clin. Chem. Clin. Biochem., 18: 521-534.

Jagota, S.K. and H.M. Dani, 1982. A new calorimetric technique for estimation of vitamin C using folin phenol reagent. Anal. Biochem., 127: 178.

Jung-Hun, S., L. Kyung-Min, N. Ji-Yoon, B. Ok-Nam, C. Seung-Min, L. Moo-Yeol and C. Jin-HO, 2007. Lead induced procoagulant activation of erythrocytes through phosphatidylserine exposure may lead to thrombotic diseases.

Kedziora-Kornatowska, K., S. Szram, T. Kornatwski, Szadujkis-Szadurski, J. Kedziora and G. Bartosz, 2003. Effect of vitamin E and vitamin C supplementation on antioxidativ state and renal glomerular basement membrane thickness in diabetic kidney. Nephron Exp Nephrol., 95(4): e134-143.

Koller, K., T. Brown, A. Spurgeon and L. Levy, 2004. Recent developmentsin low-level lead exposure and intellectual impairment in children. Environmental Health Perspectives, 112: 987-994.

Kraus, R.J. and H.E. Ganther, 1980. Reaction of cyanide with glutathione peroxidases. Biochem. Biophys. Res. Commun., 96: 1116-22.

Larsen, K., 1972. Creatinine assay by a reaction-kinetic principle. Clin. Chem. Acta., 41: 209.

Lavicoli, I., G. Carelli and E.J. Stanek, 2003. Effects of low doses of dietary lead on red blood cell production in male and female mice. Toxicol. Lett., 137: 193-199.

Leong, W.I., C.L. Bowlus, J. Tallkvist and B. Lonnerdal, 2003. DMT1 and FPN1 expression during infancy: developmental regulation of iron absorption. Am J Physiol. Gastrointest. Liver Physiol., 285: G1153-61.

Levander, O.A., S.O. Welsh and V.C. Morris, 1980. Erythrocyte deformability as affected by vitamin E deficiency and lead toxicity. Annals New York Academy of Science, 355: 227-239.

Needleman, H., 2004. Lead poisoning. Annual Review of Biochemistry, 55: 209-222.

Neeld, J.B. and W.N. Pearson, 1963. Macro and micro methods for determination of vit. A using trifluoroacetic acid. J. Nutr., 79: 454.

Ohkawa, H., W. Ohishi and K. Yagi, 1979. Lipid peroxidation (malondialdehyde). Colorimetric method. Anal. Biochem., 95: 351.

Patra, R.C., D. Swarup and S.K. Dwivedi, 2001. Antioxidant effects of alphatocopherol, ascorbic acid and l- methionine on lead induced oxidative strees to the liver, kidney and brain in rats. Toxicology, 162: 81-88.

Reed, D.J., 1990. Glutathione: toxicological implications. Annu. Rev. Pharmacol. Toxicol., 30: 603-631.

Reeves, P.G., F.H. Nielsen and V. Fahey, 1993. "AIN-93 Purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on reformulation of the AIN-76A rodent diet". J Nutr., 123: 1939-1951.

Ribarov, S.R., L.C. Benov and I.C. Benchev, 1981. The effect of lead on hemoglobin- catalyzed lipid peroxidation. Biochimica et Biophysica Acta., 664: 453-459.

Rice-Evans, C. and E. Baysal, 1987. Iron-mediated oxidative stress in erythrocytes. Biochem. J., 244: 191-196.

Roth, E.F.Jr. and H.S. Gilbert, 1984. The pyrogallol assay for superoxide dismutase: Absence of a glutathione artifact, Anal. Biochem., 137: 50.

Sandhir, R., D. Julka and K. Gill, 1994. Lipoperoxidative damage on lead exposure in rat brain and its implications on membrane bound enzymes. Pharmacol. Toxicol., 74: 66-71.

Sivaprasad, R., M. Nagaral and P. Varalakshml, 2003. Combined efficacies of lipoic acid and meso- 2,3-dimercaptosuccinic acid on lead- induced erythrocyte membrane lipid peroxidation and antioxidant atatus in rats. Hum. Exp toxicot., 22: 183-192.

Sivaprased, R., M. Nagaraj and P. Varalakshmi, 2004. Combined efficacies of lipoic acid and 2, 3 dimercapto-succinic acid against lead- induced lipid peroxidation in rat liver. J. Nutr. Biochem., 15: 18-23.

Soltaninejad, K., A. Kebriaeezadeh, B. Minaiee, S.N. Ostad, R. Hosseini and E. Azizi, 2003. Biochemical and ultra structural Exp. Toxicol. 22: 417- 423.evidences for toxicity of lead through free radicals in rat brain. Hum.