International Journal of Pharmacology

ISSN 1811-7775

science alert

ansinet
Asian Network for Scientific Information

International Journal of Pharmacology 7 (2): 217-227, 2011
ISSN 1811-7775 / DOI: 10.3923/ijp.2011.217.227

Protective Effect of Alpha-lipoic Acid Against Lead Acetate-Induced Oxidative Stress in the Bone Marrow of Rats

[1]Nagaraja Haleagrahara, [2]Tan Jackie, [3]Srikumar Chakravarthi and [4]Anupama Bangra Kulur
[1]Division of Human Biology, School of Medicine, International Medical University, Kuala Lumpur, Malaysia
[2]Division of Postgraduate Studies, International Medical University, Kuala Lumpur, Malaysia
[3]Division of Pathology, School of Medicine, International Medical University, Kuala Lumpur, Malaysia
[4]Faculty of Medicine, MAHSA University College, Kuala Lumpur, Malaysia

Abstract: The present study was designed to investigate the effects of alpha Lipoic Acid (LA) against lead acetate induced changes in free radical scavenging enzymes and lipid hydroperoxides in bone marrow of rats. Rats were exposed to lead acetate in their drinking water (500 ppm) for 14 days and alpha lipoic acid was given concurrently (25, 50 and 100 mg kg^{-1}). Blood lead levels, lipid hydroperoxides, protein carbonyl contents and oxidative marker enzymes were estimated. Lead acetate in drinking water had elicited a significant ($p<0.05$) increase in bone marrow lipid hydroperoxides (LPO) ($p<0.05$) and Protein-Carbonyl-Contents (PCC). There was a significant ($p<0.05$) decrease in total antioxidants, superoxide dismutase ($p<0.05$), glutathione peroxidase ($p<0.05$), glutathione S-transferase ($p<0.05$) and catalase levels with lead ingestion. Supplementation of alpha lipoic acid was associated with reduced serum LPO and PCC and a significant ($p<0.05$) increase in total antioxidants and antioxidant enzyme levels . There was more significant protective effect of bone marrow with 100 mg kg^{-1} b.wt. LA. The potency of alpha lipoic acid on the reversal of lead induced changes in oxidative biomarkers in bone marrow confirms the importance of lead induced oxidative stress in bone and suggests a therapeutic approach.

Key words: Lead acetate, lipid peroxidation, bone marrow, oxidative stress, alpha lipoic acid

INTRODUCTION

Lead, widely used in industry, is an environmental pollutant that can be detected in all phases of biological system and environment. It is one of the most useful metal and also the most toxic. Even though blood lead levels continue to decline over the past two decades, specific populations like infants, young children and working class are still at a higher risk (Godwin, 2001; Guidotti and Ragain, 2007). Previous study has confirmed that more than 75% of lead-exposure for the general population comes from ingestion (Patrick, 2006). Lead absorption by ingestion depends on factors such as the particle size, physical form, gastrointestinal transit time and nutritional status of a person. Lead absorption increases, with increasing age, making children and infants more vulnerable to lead intoxication (Campbell *et al*., 2004; Sanborn *et al*., 2002). Besides acute toxicity, lead has an extremely long, half-life in bone. Individuals with past exposure develop increased blood lead levels during periods of high bone turn over, or resorption, making chronic sub acute levels of lead exposure a serious health concern. Lead finds its way to the hard tissues like bone and teeth, where it accumulates, only to result in a sustained release and maintenance of an unacceptable blood lead level, many years after the exposure period (Popovic *et al*., 2004; Rio *et al*., 2001; Smith *et al*., 2008). The mechanism of lead toxicity though not explained in detail, may be due to disruption of the pro-oxidant/antioxidant balance. That leads to tissue injury via oxidative damage to critical biomolecules such as lipids, proteins and DNA. Studies also confirmed that lead inhibit circulating antioxidant enzymes, including glutathione peroxidase, catalase, super oxide dismutase (Bolin *et al*., 2006; El-Nekeety *et al*., 2009; Ercal *et al*., 2001).

Majority of the studies reported that oxidative stress contributes to the pathogenesis of lead poisoning. Reducing the possibility of lead acetate interacting with cellular metabolism of biomolecules and decreasing the reactive oxygen species generation by the use of exogenous antioxidants has received considerable attention in the recent past (Gurer and Ercal, 2000; Hsu and Guo, 2002; Patra *et al*., 2001). There has been

Corresponding Author: Nagaraja Haleagrahara, Division of Human Biology, School of Medicine, International Medical University, Knala Lumpur, 57000, Malaysia Tel: +6027317231 Fax: +60386567229

increased interest among researchers to use antioxidant nutrients and medicinal plants with antioxidant activity for protection against lead toxicity (El-Nekeety *et al*., 2009; Xu *et al*., 2005).

Alpha-Lipoic Acid (LA) is a naturally occurring compound which functions as a cofactor in several mitochondrial multienzyme complexes involved in energy production in humans and animals (Arivazhagan *et al*., 2002; Cakatay, 2006; Shay *et al*., 2009). LA is a coenzyme of pyruvate and the α-ketoglutarate dehydrogenase multienzyme complex of the tricarboxylic acid cycle and has metal chelating, free radical scavenging and antioxidant-regenerating abilities (Cakatay and Kayali, 2005; Caylak *et al*., 2008; Packer *et al*., 2001). Bioavailability studies have reported that 20-40% of lipoic acid from an oral dose appears in circulation and this rapid uptake of lipoic acid in the gastrointestinal system is followed by its transport to different tissues (Chng *et al*., 2009; Shay *et al*., 2009). Several studies have provided evidence for the protective role of lipoic acid in diseases that have been characterized by an altered state of antioxidant defense systems, like diabetes mellitus and hypertension. Alpha lipoic acid protects against oxidative stress both in peripheral tissues and central nervous system (Song *et al*., 2004; Winiarska *et al*., 2008).

There have been many reports about the lead-induced oxidative damage in peripheral tissues and central nervous system and the protective role of alpha lipoic acid against oxidative stress in various organ systems (Shay *et al*., 2009; Suh *et al*., 2004). To the best of our knowledge, there are no reports on the effect of lead acetate on perturbations in oxidative biomarkers in the bone marrow and the role of alpha lipoic acid against these biomarkers. Hence, in the present study, we investigated the beneficial effects of alpha lipoic acid on altered oxidative stress parameters and antioxidant enzyme levels in bone marrow with lead acetate treatment. We determined lipid hydroperoxides and protein carbonyl levels as an indicator of lipid peroxidation. Oxidative stress status was described by determination of total antioxidants, superoxide dismutase (SOD), glutathione peroxidase (GPx), Glutathione-S Transferase (GST) and catalase (CAT) levels.

MATERIALS AND METHODS

Chemicals: Lipid hydroperoxide, protein carbonyl content, super oxide dismutase, glutathione peroxidase, glutathione S-transferase, catalase and total antioxidant assay kits were purchased from Cayman Chemicals (Cayman Chemicals and Pierce Biotechnology, USA). Lead acetate was purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Animals: Three months old, forty male Sprague Dawley rats weighing 180-200 g were utilized for the experiments. They were fed a standard diet and had free access to water before the start of the experiment. The rats were housed in stainless steel cages in a temperature-controlled room (22±2°C) with a 12 h light and 12 h dark exposure. This study was performed in accordance with the guide lines provided for the experiments on laboratory animals and approved by the Research and Ethics Committee of the International Medical University, Kuala Lumpur.

Experimental design: Animals were divided randomly into following groups with eight animals in each group. Group 1: Control; Group 2: Rats exposed to lead acetate in drinking water (500 ppm) for 14 days; Group 3: Rats in three subgroups received lead acetate in drinking water (500 ppm) and oral alpha lipoic acid daily (25, 50 and 100 mg kg^{-1} b.wt.) for 14 days (Kishi *et al*., 1999; Thaakur and Himabindhu, 2009). Alpha lipoic acid was diluted with distilled water to the desired concentration (25, 50 and 100 mg kg^{-1} b.wt.) and the fluid was, force fed with feeding tube (0.5 mL rat/day). At the end of the 14-day experimental period, animals were sacrificed by decapitation 24 h after the last alpha lipoic acid administration. Blood was collected by cardiac puncture and stored at -20°C for blood lead estimation. The left and right femur bone of the rat was excised, the soft tissues on the bone were scrapped clean and the extreme ends of the femur were cut to reveal the marrow. A 3 mL syringe preloaded with phosphate buffer solution was used to flush the bone marrow. The flushing and washing was repeated 3-4 times and this process ensured, complete removal of bone marrow contents from the femur bone. About 5 mL of marrow solution was collected from each rat. The samples were maintained at -20°C before performing assays (not longer than 4 days). From the bone marrow samples, lipid hydroperoxides (LPO), total antioxidants, Protein Carbonyl Content (PCC), superoxide dismutase (SOD), glutathione peroxidase (GPX) and glutathione S-transferase (GST), catalase (CAT), Total Antioxidants (TA) levels and proteins were assayed using ELISA kits. Assay kits were from Cayman Chemicals (Cayman Chemicals and Pierce Biotechnology, USA). Serum samples were assayed for blood lead levels via graphite furnace atomic absorption spectrophotometry method. The research project was carried out in the research laboratory at International Medical University, Malaysia between April to November 2009.

Assays: A quantitative extraction method as provided in the ELISA kit method for LPO assay was used to extract lipid hydroperoxides into chloroform and the extract was directly used.

Lipid hydroperoxide levels were expressed as nmol mg^{-1} of protein. The carbonyl content was measured as per the kit guidelines utilizing DNPH (2, 4, dinitrophenyl hydrazine) reaction then expressed as nmol mg^{-1} protein (Janero, 1990).

Super oxide dismutase assay kit utilizes a tetrazolium salt for the detection of superoxide radicals (O_2^-) generated by xanthine oxidase and hypoxanthine. One unit of SOD is defined as the amount of enzyme necessary to exhibit 50% dismutation of superoxide radical. Superoxide dismutase levels were determined from a standard curve and expressed as U mg^{-1} of protein (Maier and Chan, 2002).

Oxidized glutathione, produced upon reduction of an organic hydroperoxide by GPX, is recycled into its reduced state by glutathione reductase enzyme and NADPH using the ELISA kit. The oxidation of NADPH to $NADP^+$ is detected by absorbance at 340 nm. Glutathione peroxidase enzyme levels were expressed as nmol mg^{-1} of protein in the sample (Ursini *et al*., 1985).

The assay kit for glutathione S-transferase, measures the total enzyme activity by measuring the conjugation of 1 chloro, 2, 4 dinitro benzene (CDNB) with reduced glutathione. The absorbance recorded at 340 nm was directly proportional to the GST activity in the sample. The bone marrow GST was expressed as μmol/h/mg protein (Mannervik *et al*., 1985).

The assay of catalase was based on the reaction of the enzyme with methanol in the presence of an optimal concentration of H_2O_2. The formaldehyde produced is measured spectrophotometrically with purpled as the chromogen. One unit is defined as the amount of enzyme that will cause the formation of 1.0 nmol of formaldehyde per minute at 25°C. The catalase levels were expressed as nmol/min/mg of protein (Wheeler *et al*., 1990).

Using the total antioxidant assay kit, aqueous and lipid soluble antioxidants were not separated and thus combined antioxidant activities of all its constituents were assessed. Bone marrow total antioxidant levels were calculated from the standard curve and expressed as μmol mg^{-1} of protein (Rice-Evans and Miller, 1994).

Pelvic bone with proximal part of the femur was excised for histopathological studies. Bone tissue was kept in 10% formalin until staining. Decalcification of the bone was done and paraffin blocks were prepared. Five-millimeter section were cut and stained with hematoxylin and eosin for histological examination.

Statistics: The results are expressed as Mean±SD. Analysis of the data was performed by one-way analysis of variance (ANOVA) and subsequent analysis was performed using the Tukey test. P value less than 0.05 was considered statistically significant.

RESULTS

Blood lead levels: There was a significant increase ($p<0.05$) in blood lead levels in the lead acetate alone group compared to other treatment groups. Animals given lead acetate alone had significantly higher ($p<0.05$) blood lead levels than lead with alpha lipoic acid groups. There was more significant decrease ($p<0.05$) in blood lead levels with 50 and 100 mg of alpha lipoic acid, compared to 25 mg dose (Fig. 1).

Total antioxidants: There was a significant ($p<0.05$) decrease in bone marrow total antioxidants after lead acetate exposure for 14 days. Concurrent treatment with alpha lipoic acid significantly ($p<0.05$) increased marrow total antioxidant levels and there was more significant increase in total antioxidants after 100 mg kg^{-1} b.wt. alpha lipoic acid. With increasing dose of alpha lipoic acid there was significant ($p<0.05$) increase in bone marrow antioxidant levels in lead with alpha lipoic acid groups (Table 1).

Lipid hydroperoxides: When compared with pair-wise manner, lead alone treated group showed a significant ($p<0.05$) increase in bone marrow lipid hydroperoxide

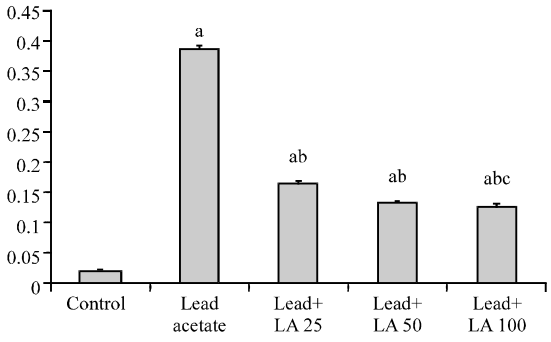

Fig. 1: Effect of alpha lipoic acid on blood lead levels (μg L^{-1}) in rats. Results are expressed as Mean±SD of eight rats per group; LA-Alpha lipoic acid, [a]Significantly different from control ($p<0.05$) [b]Significantly different from lead alone treatment group ($p<0.05$), [c]Significantly different from lead+LA group ($p<0.05$)

Table 1: Effects of lead acetate (Pb) and alpha Lipoic Acid (LA) on bone marrow oxidative parameters in rats

Parameters	Control (n = 8)	Lead acetate (n = 8)	Pb+LA (25) (n = 8)	Pb+LA (50) (n = 8)	Pb+LA (100) (n = 8)
Total antioxidants (μmol mg^{-1} protein)	5.228±0.247	2.387±0.248^{a}	3.748±0.697^{ab}	4.147±0.759^{ab}	4.829±0.227^{abc}
Lipid hydroperoxides (nmol mg^{-1} protein)	2.10±0.26	3.748±0.524^{a}	2.855±0.174^{ab}	2.778±0.144^{ab}	1.947±0.089^{bc}
Protein carbonyl content (nmol mg^{-1} protein)	12.124±0.808	22.39±0.295^{a}	18.048±0.832^{ab}	17.691±0.335^{ab}	14.529±0.523^{abc}
Superoxide dismutase (U mg^{-1} protein)	3.937±0.681	2.155±0.233^{a}	2.804±0.348^{abc}	3.441±0.815^{b}	3.809±0.578^{bc}
Glutathione peroxidase (nmol mg^{-1} protein)	8.372±0.189	3.972±0.371^{a}	7.645±0.005^{ab}	7.873±0.951^{ab}	8.019±0.613^{bc}
Glutathione S-Transferase (μmol/h/mg protein)	4.575±0.389	2.249±0.485^{a}	3.112±0.034^{ab}	3.813±0.084^{abc}	4.194±0.213^{abc}
Catalase (nmol/min/mg of protein)	4.816±0.185	2.714±0.428^{a}	3.019±0.015^{ab}	3.867±0.024^{ab}	4.216±0.019^{abc}

Values are Mean±SD. Within each row, means superscript with different letter(s) is significantly different ($p<0.05$). ^{a}Significantly different from control ($p<0.05$); ^{b}Significantly different from lead alone treatment group ($p<0.05$); ^{c}Significantly different from lead+LA group ($p<0.05$)

levels compared to control group. Alpha lipoic acid treatment with concurrent lead acetate exposure resulted in significant ($p<0.05$) decrease in LPO level, compared to lead alone group. Alpha lipoic acid at all three doses decreased LPO levels significantly ($p<0.05$). More significant ($p<0.05$) decrease in LPO was recorded from 100 mg of alpha lipoic acid treatment compared to other two doses. In the concurrent treatment groups, alpha lipoic acid had successfully brought back the LPO levels to near normal control levels ($p<0.05$) (Table 1).

Protein carbonyl content: A significant ($p<0.05$) increase in bone marrow protein carbonyl contents were recorded after lead treatment. More than 80% increase in protein carbonyl content was seen in lead acetate treated group. The level of PCC decreased significantly ($p<0.05$) with concurrent alpha lipoic acid, it did not significantly decrease below the control levels (Table 1). There was statistically significant ($p<0.05$) difference in protein carbonyl contents between alpha lipoic acid with lead acetate groups and more significant decrease in PCC was seen in 100 mg kg^{-1} b.wt. group (Table 1).

Superoxide dismutase: Serum superoxide dismutase decreased significantly ($p<0.05$) after lead treatment for 14 days, but treatment with alpha lipoic acid significantly ($p<0.05$) increased the SOD levels after 14 days. Lead with alpha lipoic acid at 100 mg kg^{-1}, increased the SOD levels more than lead alone group ($p<0.05$). With increasing dose of alpha lipoic acid treatment, bone marrow SOD level increased significantly ($p<0.05$) and reached near normal level at 100 mg kg^{-1} b.wt. (Table 1).

Glutathione peroxidase: Table 1 shows the bone marrow glutathione peroxidase level with alpha lipoic acid and lead acetate treatment. Lead acetate for 14 days in drinking water decreased the glutathione peroxidase levels significantly ($p<0.05$) and treatment with alpha lipoic acid along with lead was able to increase the glutathione peroxidase levels. Bone marrow GPX level was significantly higher ($p<0.05$) than lead alone group, but the level was lower than control group ($p<0.05$). No significant difference was seen in SOD levels with three dose of alpha lipoic acid in the concurrent treatment groups.

Glutathione S-transferase: There was a significant ($p<0.05$) decrease in bone marrow glutathione S-transferase levels in lead treatment group when compared to control rats. Alpha lipoic acid treatment with concurrent lead exposure showed a significant increase ($p<0.05$) in GST level compared to lead alone group. Glutathione S-transferase level increased significantly ($p<0.05$) in 50 and 100 mg of alpha lipoic acid and this level was significantly higher than 50 mg group. Though alpha lipoic acid treatment significantly ($p<0.05$) increased the bone marrow GST in the treatment groups, the level was significantly lower in 25 mg than control rats (Table 1).

Catalase: Bone marrow catalase levels decreased more than 50% after lead acetate exposure for two weeks, compared to control levels ($p<0.05$). Treatment with alpha lipoic acid during lead exposure resulted in significant ($p<0.05$) increase in catalase levels. The levels were significantly ($p<0.05$) more than lead alone group. Statistical analyses between alpha lipoic acid in three different doses showed a significant ($p<0.05$) increase in bone marrow catalase concentrations in 100 mg kg^{-1} b.wt. group. With 100 mg kg^{-1} b.wt. alpha lipoic acid, bone marrow catalase level was brought to near normal level (Table 1).

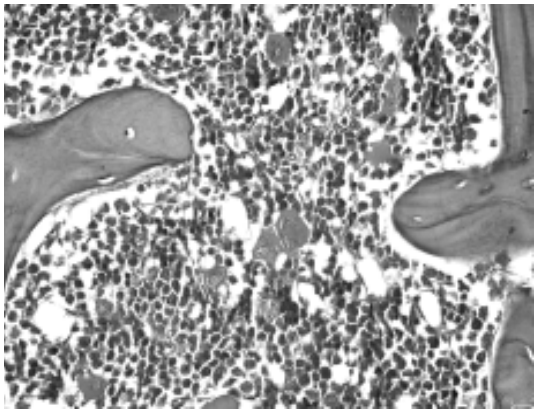

Fig. 2A: Photomicrograph of bone marrow from control rat showing normal bone histology with healthy marrow, normal bony spicules and adequate marrow spaces (H and E, X400)

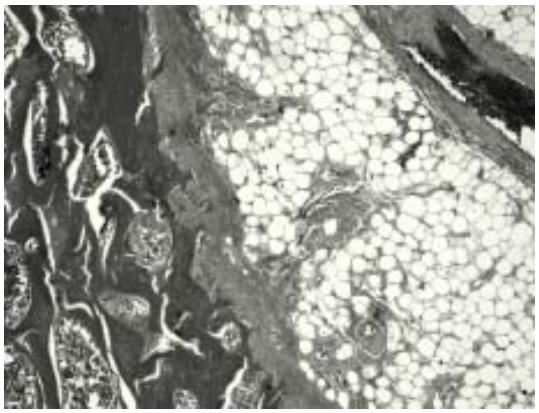

Fig. 2B: Photomicrograph of bone marrow from rat treated with lead acetate alone for 14 days, showing thickening of bone, irregular bony spicules, mild osteoporotic changes (H and E X400)

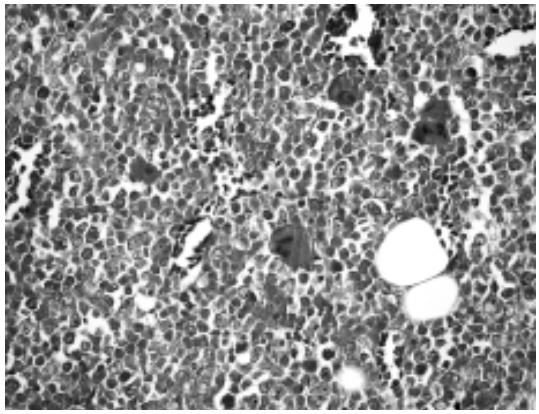

Fig. 2C: Photomicrograph of bone marrow from rat treated with Lead with Alpha Lipoic Acid (25 mg) showing mild marrow hyperplasia with hypoplasia, adequate irregular marrow spaces (H and E X400)

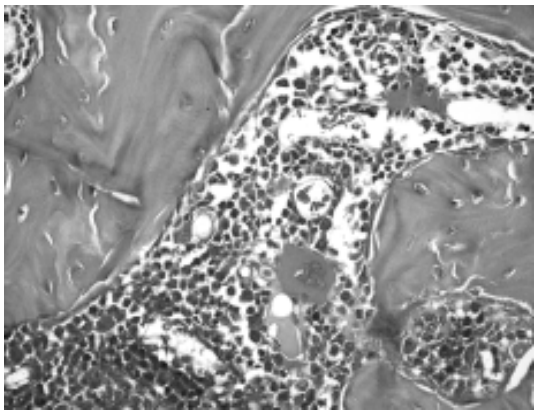

Fig. 2D: Photomicrograph of bone marrow rat treated with Lead with Alpha Lipoic Acid (50mg) showing adequate marrow spaces (H and E X400)

Histopathological changes: The control group depicted a healthy cortical bone, with normal bone marrow. No osteoporotic or osteosclerotic changes were seen in bone histology (Fig. 2A). Rats treated with lead acetate alone for 14 days showed significant toxic changes in bone marrow suggestive of lead-induced damage. There were areas of compressed marrow material, showing very little hematopoietic tissue and increased amount of fat cells. Significant marrow hyperplasia and early osteoporotic changes were also seen (Fig. 2B). Rats treated with lead acetate with 25 mg of alpha lipoic acid have shown little improvement from the lead acetate damage and there was only mild bone marrow hyperplasia with irregular marrow spaces (Fig. 2C). Alpha lipoic acid of 50 mg with lead acetate showed significant improvement in bone marrow histology with adequate marrow spaces with good myeloid to erythroid ratio (Fig. 2D). There was very minimum evidence of any bone marrow damage 100 mg of alpha lipoic acid treatment, where there were scattered fat cells, with healthy marrow and a good hematopoietic tissue (Fig. 2E). Active proliferation of erythroid and myeloid cells were observed in many rats in this group.

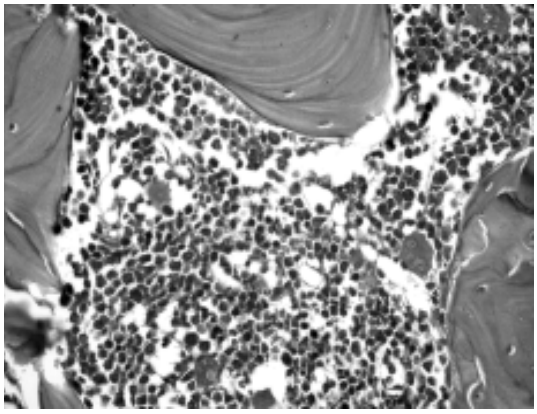

Fig. 2E: Photomicrograph of bone marrow rat treated with Lead with Alpha Lipoic Acid (100 mg) showing healthy marrow, adequate marrow spaces and evenly scattered fat cells (H and E, X400)

DISCUSSION

Lead toxicity has been among the most studied health problems in the recent years. Although the affinity of lead for the bone has been widely recognized for many years. clinical and experimental evaluations of lead toxicity in bone are relatively rare and less sophisticated in approach than the study of lead-induced toxic damage in other target organs such as liver and the nervous system (Bellinger, 2008; Upasani *et al*., 2001). Despite the large body of evidence about the lead induced organ damage, there are no known mechanisms of lead toxicity explaining some of the toxic effects of lead. Lead is known to cause oxidative damage in various tissues by bringing about imbalance in the generation and removal of reactive oxygen species (Gurer and Ercal, 2000; Hamadouche *et al*., 2009). Although the exact mechanisms by which lead induces oxidative stress in various tissues are not completely understood, evidence indicates that multiple mechanisms may be involved.

The result of the present study showed that there was a significant increase in blood lead levels following lead acetate in drinking water for two weeks. Lead acetate absorbed from gastrointestinal tract is carried via blood, mainly in the erythrocytes to the soft tissues and also to bone (Freeman, 1970). In the present study, lead acetate exposure in drinking water for 14 days resulted in severe oxidative stress in bone marrow. The dose of lead acetate used in this research was based on previous literature (Bokara *et al*., 2009). There was increase in lipid hydroperoxide and protein carbonyl content in bone marrow and decrease in total antioxidants and antioxidant enzymes like super oxide dismutase, glutathione peroxidase, glutathione S-transferase and catalase levels. This observation supports the findings of several earlier studies, which reported alterations in antioxidant enzyme activities in lead exposed animals (Bolin *et al*., 2006; El-Nekeety *et al*., 2009; McGowan and Donaldson, 1986) and workers (Solliway *et al*., 1996; Sugawara *et al*., 1991). The toxicant chemicals induce perturbations in the physiological and biochemical state, which affects the enzyme activity. It then causes distortions in the cell organelles, which may lead to alterations in various enzyme concentrations (Vinodhini and Narayanan, 2009; Wollin and Jones, 2003). The lead-induced toxicity stimulates the oxidative stress and the antioxidant enzymes levels are increased as a defense mechanism. Our findings support the involvement of oxidative stress in the pathophysiology of lead toxicity in bone marrow. But it is not clear whether the changes in marrow antioxidant enzymes are the cause of oxidative damage or a consequence of it.

Lead is known to cause oxidative damage in various peripheral organs by enhancing lipid peroxidation (Gurer *et al*., 1998; Hamadouche *et al*., 2009; Halliwell and Gutteridge, 1989; Landrigan *et al*., 2000). Lipid hydroperoxides are formed due of oxidation of lipid and cholesterol containing cellular molecules like cell membrane phospholipids, lipoproteins, glycolipid, cholesterol and other lipid-containing structures (Porter *et al*., 1995). The oxidation is usually caused by ROS like oxyl radicals, peroxyl radicals and hydroxyl radicals (Hsu and Guo, 2002). Bone marrow LPO levels increased significantly with lead acetate ingestion and this could be due to lead induced inhibition of radical scavenging enzymes like GST and SOD. These indirectly cause ROS to accumulate in bone marrow and cause increased oxidation (Ribarov and Bochev, 1982). Enzymes like GPx which converts peroxides into alcohol and water (Ursini *et al*., 1985) were also inhibited as observed in the study, pointing out that LPO once formed is not readily removed during lead toxicity. The reason for LPO increase could also be a combinational inhibitory effect of all the antioxidant enzymes (GPX, SOD, CAT and GST) as observed in the results. Protein carbonyls are formed when lysine, arginine, proline, histidine side chains of proteins are oxidized (Halliwell, 1996; Halliwell and Gutteridge, 1989). Increased protein carbonyl contents in bone with lead ingestion could be due to an increased ROS level in the marrow tissue.

There was a significant reduction in reduced glutathione peroxidase level in the bone marrow with lead acetate ingestion. This might be due to lead's

displacement of the selenocysteine group from the active site in the marrow tissue. The selenium containing functional group at the active site of Gpx is responsible for it catalytic functions (Forstrom *et al*., 1978). Glutathione S-transferase catalyses a conjugation reaction, that adds a reduced glutathione to electrophilic groups on the molecule. This makes the original molecule, more soluble (Habig *et al*., 1974; Leaver and George, 1998). GST is also neutralizing oxyl radicals, peroxyl radicals and hydroxyl radicals. The significant reduction in GST levels observed after lead exposure could be due to lead binding irreversibly with-SH groups on proteins, rendering them useless (Gurer and Ercal, 2000; Hsu and Guo, 2002; Newairy and Abdou, 2009).

Superoxide dismutase is a group of enzymes which catalyzes the conversion of superoxide anion (O_2^-) to hydrogen peroxide (H_2O_2). The functional groups of the few types of SOD consist of transitional metals like copper/zinc, manganese and iron (Maier and Chan, 2002; Sandstrom *et al*., 1994). Present results showed that there was a significant reduction in super oxide dismutase level in the bone marrow after two weeks of lead exposure, confirming the oxidant effects of lead acetate in marrow tissue. The balance between the production of oxidants and removal of these oxidants by antioxidant enzymes determines the extent of oxidative damage in tissues. In the present study, the activities of SOD, GPx, CAT and GST antioxidants were reduced by lead acetate, thus rendering the bone marrow to the peroxidative damage.

In addition to acute toxicity, lead is known to have extremely long half life in bone (Pounds *et al*., 1991). Individuals with past exposure develop increased blood lead levels during periods of high bone turn over or resorption, making chronic, sub acute levels of lead exposure a serious health concern. Lead finds its way to the hard tissues like bone and teeth, where it accumulates, only to result in a sustained release and maintenance of unacceptable blood lead levels, many years after exposure period. Lead is known to affect osteoclasts and chondrocytes and has been associated with osteoporosis (Schwartz *et al*., 1986; Shukla *et al*., 1987; Pounds *et al*., 1991). However, its effects on free radical generation and oxidative damage in bone marrow have not been studied in detail. Lead has been reported to damage vital organs like liver, kidney and brain and suppresses cellular processes. Beside its competition with essential metals and its high affinity to thiol groups in proteins, the production of free radicals (Hsu *et al*., 1997) as well as decreasing circulating antioxidants and increasing lipid peroxidation have been reported as lead induced toxic effects (El-Sokkary *et al*., 2003; El-Missiry, 2000; Othman *et al*., 2004).

Alpha lipoic acid (1, 2-dithiolane-3-pentanoic acid), plays an essential role in mitochondrial dehydrogenase reactions, and is present in all kinds of prokaryotic and eukaryotic cells and has recently gained significant interest as an antioxidant (Caylak *et al*., 2008; Shay *et al*., 2009). In the present study, administration of alpha lipoic acid at three different doses along with lead markedly hampered lead-induced toxicity on all studied parameters of the bone marrow. Alpha Lipoic acid co-treatment significantly inhibited the levels of lipid hydroperoxides, protein carbonyl contents and, stimulated antioxidant enzyme activities like SOD, GPx, GST and CAT. Concurrent treatment also recovered the histopathological changes in the bone marrow.

The observed increase in total antioxidants and antioxidant enzyme levels with alpha lipoic acid treatment to lead ingesting rats could be due to the antioxidant effects of LA. LA could either mitigate antioxidant enzyme consumption by acting as an alternate ROS scavenger or increase enzyme levels by stimulating its biosynthesis with an unknown mechanism. Alpha lipoic acid is characterized by high reactivity toward reactive oxygen species and its capability of increasing tissue levels of antioxidant enzymes (Biewenga *et al*., 1997; Packer *et al*., 1995; Shay *et al*., 2008). It has been demonstrated that LA reduces oxidative stress in healthy adults and diabetic patients by decreasing significantly lipid-hydroperoxide formation (Packer *et al*., 2001; Smith *et al*., 2004). The protective action of alpha lipoic acid against lipid peroxidation as a factor modifying membrane organization may due to alpha lipoic acid's ability to scavenge the free radicals, which are produced during the peroxidation of lipids. Since membrane functions and structure are influenced by proteins in membranes and lead acetate is known to damage thiol proteins (El-Sokkary *et al*., 2003), it is possible that the protective action of LA to membrane damage induced by lead may be related partially to the ability to prevent protein damage. Several studies have reported that alpha lipoic acid scavenges ROS and chelate transition metals (Ou *et al*., 1995; Packer *et al*., 2001; Suzuki *et al*., 1991). Therefore, it is reasonable to presume that they oppose the lipid peroxidation process that is known to be triggered by ROS in bone marrow.

In histopathological study, there was a decreased myeloid: erythroid ratio, osteosclerosis, osteoporotic changes and reduced intervening marrow spaces in the

lead ingested group in bone marrow. Areas of fibrosis and areas of fat cell congestion were seen indicating lead-induced damage. (Othman *et al*., 2004). Studies done by El-Ashmawy *et al.* (2006) reported that lead toxicity causes a weak clastogenicity effect on rat bone marrow cells. They observed a reduced number of dividing cells, increased number of abnormal cells and an increased frequency of chromosomal aberrations. Increased generation of reactive oxygen species could have been, the cause for observed changes (Hamadouche *et al*., 2009). With alpha lipoic acid treatment there was good regenerative changes observed and there was only mild degree of marrow hyperplasia which also showed that alpha lipoic acid counteracted lead-induced clastogenic activities in lead treatment groups by inducing increased marrow growth.

Despite its antioxidant effects, LA was also reported to form stable complexes with Mn^{+2}, Cu^{+2}, Zn^{+2} (Sigel *et al*., 1978), as well as chelating cadmium and iron (Ou *et al*., 1995; Shay *et al*., 2009). *In vitro* studies showed that LA preferentially binds to Cu^{2+}, Zn^{2+} and Pb^{2+}, but cannot chelate Fe^{3+}, while DHLA forms complexes with Cu2+, Zn2+, Pb2+, Hg^{2+} and Fe^{3+} (Suh *et al*., 2005). Dihydrolipoic acid (DHLA), rapidly formed by the reduction of LA in cells, has two sulphydryl groups that support the promising chelating effect for lead. In the present study, treatment with alpha lipoic acid at three doses showed a significant decreased in blood lead levels. These results, confirm that LA is capable of removing lead from the bloodstream and target organs. Thus, the present study confirms alpha lipoic acid as an ideal antioxidant against lead induced bone marrow toxicity because; it has the ability to scavenge reactive species; it can regenerate other antioxidants such as SOD, GST, GPx and CAT from their radical or inactive forms; and it has lead chelating activity.

Thus, in conclusion, this study showed that exposure to lead acetate caused a marked increase in lipid peroxidation and a reduction in free radical scavenging enzymes in bone marrow. While, concurrent treatment of alpha lipoic acid, with lead acetate minimized its toxic effects in bone tissues. The protective effect of alpha lipoic acid may be due to its free radical scavenging activities in bone marrow. These observations clearly exemplified that alpha lipoic acid is quite useful and suitable candidate against bone marrow lead toxicity. Though the exact mechanism underlying the protective effect of alpha lipoic acid against lead is not known, further experimental studies are to be conducted to confirm such effects.

ACKNOWLEDGMENT

The research is funded by International Medical University, Kuala Lumpur, Malaysia, research grant.

REFERENCES

Arivazhagan, P., S. Shila, S. Kumaran and C. Panneerselvam, 2002. Effect of DL-alpha-lipoic acid on the status of lipid peroxidation and antioxidant enzymes in various brain regions of aged rats. Exp. Gerontol., 37: 803-811.

Bellinger, D.C., 2008. Very low lead exposures and children's neurodevelopment. Curr. Opin. Pediatr., 20: 172-177.

Biewenga, G.P., G.R.M.M. Haenen and A. Bast, 1997. The pharmacology of the antioxidant lipoic acid. Gen. Pharmacol., 29: 315-331.

Bokara, K.K., I. Blaylock, S.B. Denise, R. Bettaiya, S. Rajanna and P.R. Yallapragada, 2009. Influence of lead acetate on glutathione and its related enzymes in different regions of rat brain. J. Applied Toxicol., 29: 452-458.

Bolin, C.M., R. Basha, D. Cox, N.H. Zawia, B. Maloney, D.K. Lahiri and F. Cardozo-Pelaez, 2006. Exposure to lead and the developmental origin of oxidative DNA damage in the aging brain. FASEB J., 20: 788-790.

Cakatay, U., 2006. Pro-oxidant actions of alpha-lipoic acid and dihydrolipoic acid. Med. Hypotheses, 66: 110-117.

Cakatay, U.R. and R. Kayali, 2005. Plasma protein oxidation in aging rats after alpha-lipoic acid administration. Biogerontology, 6: 87-93.

Campbell, J.R., R.N. Rosier, L. Novotny and J.E. Puzas, 2004. The association between environmental lead exposure and bone density in children. Environ. Health. Perspect., 112: 1200-1203.

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

Chng, H.T., L.S. New, A.H. Neo, C.W. Goh, E.R. Browne and E.C. Chan, 2009. Distribution study of orally administered lipoic acid in rat brain tissues. Brain Res., 1251: 80-86.

El-Ashmawy, I.M., K.M. Ashry, A.F. El-Nahas and O.M. Salama, 2006. Protection by turmeric and myrrh against liver oxidative damage and genotoxicity induced by lead acetate in mice. Basic Clin. Pharmacol. Toxicol., 98: 32-37.

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

El-Nekeety, A.A., A.A. El-Kady, M.S. Soliman, N.S. Hassan and M.A.A. Wahhab, 2009. Protective effect of *Aquilegia vulgaris* (L.) against lead acetate-induced oxidative stress in rats. Food Chem. Toxicol., 47: 2209-2215.

El-Sokkary, G.H., E.S. Kamel and R.J. Reiter, 2003. Prophylactic effect of melatonin in reducing lead-induced neurotoxicity in the rat. Cell. Mol. Lett., 8: 461-470.

Ercal, N., H. Gurer-Orhan and N. Aykin-Burns, 2001. Toxic metals and oxidative stress part I: Mechanisms involved in metal-induced oxidative damage. Curr. Top. Med. Chem., 1: 529-539.

Forstrom, J.W., J.J. Zakowski and A.L. Tappel, 1978. Identification of the catalytic site of the rat liver glutathione peroxidase as selenocysteine. Biochem., 17: 2639-2644.

Freeman, R., 1970. Chronic lead poisoning in children: A review of 90 children diagnosed in Sydney, 1948-1967. 2. Clinical features and investigations. Med. J. Aust., 1: 648-681.

Godwin, H.A., 2001. The biological chemistry of lead. Curr. Opin. Chem. Biol., 5: 223-227.

Guidotti, T. and L. Ragain, 2007. Protecting children from toxic exposure: Three strategies. Ped. Clin. North Am., 54: 227-235.

Gurer, H. and N. Ercal, 2000. Can antioxidants be beneficial in the treatment of lead poisoning. Free Radic. Biol. Med., 29: 927-945.

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. Toxicol., 128: 181-189.

Habig, W.H., M.J. Pabst and W.B. Jakoby, 1974. Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. J. Biol. Chem., 249: 7130-7139.

Halliwell, B. and J.M.C. Gutteridge, 1989. Protection against Oxidants in Biological Systems: The Superoxide Theory of Oxygen Toxicity. In: Free Radical in Biology and Medicine, Halliwell, B. and J.M.C. Gutteridge (Eds.). Clarendon Press, Oxford, pp: 86-123.

Halliwell, B., 1996. Commentary oxidative stress, nutrition and health. Experimental strategies for optimization of nutritional antioxidant intake in humans. Free Radical Res., 25: 57-74.

Hamadouche, N.A., M. Slimani, B. Merad-Boudia and C. Zaoui, 2009. Reproductive toxicity of lead acetate in adult male rats. Am. J. Sci. Res., 3: 38-50.

Hsu, P.C., M.Y. Liu, C.C. Hsu, L.Y. Chen and G.Y. Leon, 1997. Lead exposure causes generation of reactive oxygen species and functional impairment in rat sperm. Toxicology, 122: 133-143.

Hsu, P.C. and Y.L. Guo, 2002. Antioxidant nutrients and lead toxicity. Toxicol., 180: 33-44.

Janero, D.R., 1990. Malondialdehyde and thiobarbituric acid-reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury. Free Rad. Biol. Med., 9: 515-540.

Kishi, Y., J.D. Schmelzer, J.K. Yao, P.J. Zollman, K.K. Nickander, H.J. Tritschler and P.A. Low, 1999. Alpha-lipoic acid: Effect on glucose uptake, sorbitol pathway and energy metabolism in experimental diabetic neuropathy. Diabetes, 48: 2045-2051.

Landrigan, P.J., P. Boffetta and P. Apostoli, 2000. The reproductive toxicity and carcinogenicity of lead: A critical review. Am. J. Ind. Med., 38: 231-243.

Leaver, M.J. and S.G. George, 1998. A piscine glutathione S-transferase which efficiently conjugates the end-products of lipid peroxidation. Mar. Environ. Res., 46: 71-74.

Maier, C.M. and P.H. Chan, 2002. Role of superoxide dismutases in oxidative damage and neurodegenerative disorders. Neuroscientist, 8: 323-334.

Mannervik, B., P. Alin, C. Guthenberg, H. Jensson, M.K. Tahir, M. Warholm and H. Jornvall, 1985. Identification of three classes of cytosolic glutathione transferase common to several mammalian species:correlation between structural data and enzymatic properties. Proc. Natl. Acad. Sci. USA., 82: 7202-7206.

McGowan, C. and W.E. Donaldson, 1986. Changes in organ non-protein sulfhydryl and glutathione concentrations during acute and chronic administration of inorganic lead to chicks. Biol. Trace. Elem. Res., 10: 37-46.

Newairy, A.A. and H.M. Abdou, 2009. Protective role of flax lignans against lead acetate induced oxidative damage and hyperlipidemia in rats. Food. Chem. Toxicol., 47: 813-818.

Othman, A.I., S. Al Sharawy and M.A. El-Missiry, 2004. Role of melatonin in ameliorating lead induced haematotoxicity. Pharmacol. Res., 50: 301-307.

Ou, P., H.J. Tritschler and S.P. Wolff, 1995. Thioctic acid: A therapeutic metal-chelating antioxidant. Biochem. Pharmacol., 50: 123-126.

Packer, L., E.H. Witt and H.J. Tritschler, 1995. Alpha-lipoic acid as a biological antioxidant. Free Radical Biol. Med., 19: 227-250.

Packer, L., K. Kraemer and G. Rimbach, 2001. Molecular aspects of lipoic acid in the prevention of diabetes complications. Nutr., 17: 888-895.

Patra, R.C., D. Swarup and S.K. Dwivedi, 2001. Antioxidant effects of a-tocopherol, ascorbic acid and L-methionine on lead induced oxidative stress to the liver, kidney and brain in rats. Toxicol., 162: 81-88.

Patrick, L., 2006. Lead toxicity, a review of the literature. Part 1: Exposure, evaluation and treatment. Alt. Med. Rev., 11: 2-22.

Popovic, M., F.E. McNeill, C.E. Webber and D.R. Chettle, 2004. The effect of lead in bone densitometry. Nuclear Instruments Methods Physics Res. B, 213: 599-602.

Porter, N.A., S.E. Caldwell and K.A. Mills, 1995. Mechanisms of free radical oxidation of unsaturated lipids. Lipids, 30: 277-290.

Pounds, J.G., J.G. Long and J.F. Rosen, 1991. Cellular and molecular toxicity of lead in bone. Environ. Health. Perspect., 91: 17-32.

Ribarov, S.R. and P.G. Bochev, 1982. Lead–hemoglobin interaction as a possible source of reactive oxygen species: A chemiluminescent study. Arch. Biochem. Biophys., 213: 288-292.

Rice-Evans, A. and N. Miller, 1994. Total antioxidant status in plasma and body fluids. Meth. Enzym., 234: 279-293.

Rio, B., R. Froquet and D. Parent-Massin, 2001. *In vitro* effect of lead acetate on human erythropoietic progenitors. Cell. Biol. Toxicol., 17: 41-50.

Sanborn, M.D., A. Abelsohn, M. Campbell and E. Weir, 2002. Identifying and managing adverse environmental health effects: 3. Lead exposure. Can. Med. Asso. J., 166: 1287-1292.

Sandstrom, J., P. Nilsson, K. Karlsson and S.L. Marklund, 1994. 10-Fold increase in human plasma extracellular superoxide dismutase content caused by a mutation in herparin-binding domain. J. Biol. Chem., 269: 19163-19166.

Schwartz, J., C.R. Angle and H. Pitcher, 1986. Relationship between childhood blood-lead levels and stature. Pediatrics, 77: 281-288.

Shay, K.P., R.F. Moreau, E.J. Smith, A.R. Smith and T.M. Hagen, 2009. Alpha-lipoic acid as a dietary supplement: Molecular mechanisms and therapeutic potential. Biochem. Biophys. Acta, 1790: 1149-1160.

Shay, P.K., R.F. Moreau, E.J. Smith and T.M. Hagen, 2008. Is alpha-lipoic acid a scavenger of reactive oxygen species *in vivo*. Evidence for its initiation of stress signaling pathways that promote endogenous antioxidant capacity. IUBMB Life, 60: 362-367.

Shukla, R., R.L. Bornschein, K.N. Dietrich, T. Mitchell and J. Grote *et al*., 1987. Effects of Fetal and early Postnatal Lead Exposure on Child's Growth in Stature. The Cincinnati Lead Study. In: International Conference. Heavy Metals in the Environment, Lindber S.E. and T.C. Hugchison (Eds.). Vol. 1, CEP Consultants, Edinburgh, pp: 210-212.

Sigel, H., B. Prijs, D.B. McCormick and J.C.H. Shih, 1978. Stability and structure of binary and ternary complexes of α-lipoate and lipoate derivatives with Mn^{2+}, Cu^{2+} and Zn^{2+} in solution. Arch. Biochem. Biophys., 187: 208-214.

Smith, A.R., S.V. Shenvi, M. Widlansky, J.H. Suh and T.M. Hagen, 2004. Lipoic acid as a potential therapy for chronic diseases associated with oxidative stress. Curr. Med. Chem., 11: 1135-1146.

Smith, Jr. D.M., H.W. Mielke and J.B. Heneghan, 2008. Subchronic lead feeding study in male rats. Arch. Environ. Contam. Toxicol., 55: 518-528.

Solliway, B.M., A. Schaffer, H. Pratt and S. Yannai, 1996. Effect of exposure to lead on selected biochemical and hematological variables. Pharmacol. Toxicol., 78: 18-22.

Song, K.H., W.J. Lee, J.M. Koh, H.S. Kim and J.Y. Youn *et al*., 2004. Alpha-lipoic acid prevents diabetes mellitus in diabetes-prone obese rats. Biochem. Biophys. Res. Commun., 326: 197-202.

Sugawara, E., K. Nakamura, T. Miyake, A. Fukumura and Y. Seki, 1991. Lipid peroxidation and concentration of glutathione in erythrocytes from workers exposed to lead. Br. J. Ind. Med., 48: 239-242.

Suh, J.H., H. Wang, R.M. Liu, J. Liu and T.M. Hagen, 2004. (R)-alpha-lipoic acid reverses the age-related loss in GSH redox status in post-mitotic tissues: evidence for increased cysteine requirement for GSH synthesis. Arch. Biochem. Biophys., 423: 126-135.

Suh, J.H., R. Moreau, S.H. Heath and T.M. Hagen, 2005. Dietary supplementation with (R)- alpha-lipoic acid reverses the age-related accumulation of iron and depletion of antioxidants in the rat cerebral cortex. Redox. Rep., 10: 52-60.

Suzuki, Y.J., M. Tsuchiya and L. Packer, 1991. Antioxidant activities of dihydrolipoic acid and its structural homologues. Free Radic. Res. Commun., 18: 115-122.

Thaakur, S. and G. Himabindhu, 2009. Effect of Aipha- lipoic acid on the tardive dyskinesia and oxidative stress induced by haloperidol in rats. J. Neural Transmission, 116: 807-814.

Upasani, C.D., A. Khera and R. Balaraman, 2001. Effect of lead with Vitamins E, C, or Spirulina on malondialdehyde: Conjugated dienes and hydroperoxides in rats. Ind. J. Exp. Biol., 39: 70-74.

Ursini, F., M. Maiorino and C. Gregolin, 1985. The selenoenzyme phospholipid hydroperoxide glutathione peroxidase. Biochim. Biophys. Acta., 839: 62-70.

Vinodhini, R. and M. Narayanan, 2009. Biochemical changes of antioxidant enzymes in common carp (*Cyprinus carpio* L.) after heavy metal exposure. Turk. J. Vet. Anim. Sci., 33: 1-6.

Wheeler, C.R., J.A. Salzman, N.M. Elsayed, S.T. Omaye and D.W. Jr. Korte, 1990. Automated assays for superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase activity. Anal. Biochem., 184: 193-199.

Winiarska, K., D. Malinska, K. Szymanski, M. Dudziak and J. Bryla, 2008. Lipoic acid ameliorates oxidative stress and renal injury in alloxan diabetes rabbits. Biochimie, 90: 450-459.

Wollin, S.D. and P.J. Jones, 2003. Alpha-lipoic acid and cardiovascular disease. J. Nutr., 133: 3327-3330.

Xu, Y., G. Li, C. Han, L. Sun, R. Zhao and S. Cui, 2005. Protective effects of *Hippophae rhamnoides* L. juice on lead-induced neurotoxicity in mice. Biol. Pharma. Bull., 28: 490-494.