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EVALUATION OF EFFECTS OF LEAD, ALCOHOL AND VITAMIN E ON LIPID PEROXIDATION ON BRAIN TISSUE

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ABSTRACT

In vitro evaluation of lipid peroxidation activity on brain tissue of rats treated with lead, alcohol and vitamin E individually and in combination were evaluated at two, four and eight weeks of treatment . In eight weeks of treatment, maximum effect of lead and alcohol was recorded and the MDA(Malondialdehyde) value was58.74nmol/gram of brain tissue in lead, 69.74nmol/gram in alcohol and 86.68nmol/gram in lead with alcohol treated rats. In Vitamin treated rats, the MBA value was 21.32nmol/gram and 28.08nmol/gram in lead with vitamin E treated rats and in alcohol with vitamin E treated rats, the MDA value was 38.06nmol/gram of brain tissue. In combination of lead, alcohol and vitamin E, the MDA value was 39.60nmol/gram tissue.

Key words: lipid peroxidation, Lead, Alcohol, Vitamin E, Brain tissue

INTRODUCTION

Lead is a polluting agent of the ecosystem and is introduced into the food chain by various mechanisms. Lead poisoning is, and for centuries has been, one of the most significant preventable causes of neurological morbidity from an environmental toxin. As a heavy metal, lead is ubiquitous in our environment, yet it has no physiologic role in biological systems. (Djebli N et al. 2004). Lead is an ubiquitous element in the environment, it is used in many industrial activities including mining, refining and producing lead – acid batteries (Flora J etal. 2004). Although this heavy metal is less widely used today, it remains a significant public health problem. Animals may be exposed to lead via contaminated food or water and fuel additives (Goyer RA , 1989). The alimentary and respiratory tract are the major routes of lead entry into the body (Fischbein A, 1999). Once the lead is in the bloodstream, it is distributed into soft and hard tissues (Gerhardsson L et al. 1995). Milk is the most important food source for newborn, however, also be a pathway of maternal excretion of toxic elements such as lead, and these toxins impact most severely on the newborn at a time of rapid development of the central nervous system (Astrup JAS, 1991). The mechanisms of lead-related pathologies, many of which are a direct result of the oxidant effect of lead on tissues and cellular components, may be mitigated by improving the cellular availability of antioxidants. N-acetylcysteine (NAC), zinc, vitamins B6, C and E, selenium, taurine, and alpha-lipoic acid have been shown, in a number of animal studies, to interrupt or minimize the damaging effects of lead and improve the effects of pharmaceutical chelating agents(Lyn PND, 2006). Oxidative stress contributed to suppressed serum immunoglobulin levels during lead intoxication in

lead-exposed rats with blood lead level of 45.0 µgm/dl (Ercal N et al. 2000). This evidence implicating oxidative stress in lead-induced immunosuppression *in vivo* has opened new avenues for investigation of the latter's mechanisms. The mechanisms for lead-induced oxidative stress include the effect of lead on membrane, DNA, and antioxidant defense systems of cells. On cell membrane, the presence of double bonds in the fatty acid weakens the C-H bonds on the carbon atom adjacent to the double bonds and makes H removal easier. Therefore, fatty acids containing zero to two double bonds are more resistant to oxidative stress than the polyunsaturated fatty acids with more than two double bonds (Halliwell B and Gutteridge JMC, 1989). In the present study, effect of lead, alcohol and vitamin E were tested on lipid peroxidation activity.

MATERIALS AND METHODS

***1. Test Animal*:** Male Sprague Dawley rats weighing around 150 grams at the age of three months old were used in this study. The animals were housed in polypropylene cages under hygienic conditions and feedings were done using rat pellet diet (Hindustan Lever Limited) and water *ad libitum*. Permission was taken from ethical committee to conduct experiment with its reference number CPCSEA/CH/org/2000/241.

1.1.Treatment of rats with Lead, Alcohol and Vitamin E: The test animals were divided into eight groups and each group consists of six animals. Group I acts as control receiving water. Group II were treated with lead acetate at 160mg/lt concentration dissolved in water. Group III animals were treated with 10% alcohol. Group IV animals were treated with 160 mg/lt concentration of lead acetate and 10% alcohol. Group V animals served as control treated with Vitamin E/kg diet. Group VI animals were treated with lead acetate at 160mg/lt concentration dissolved in water and Vitamin E/kg diet. Group VII animals were treated with 10% alcohol and Vitamin E/kg diet. Group VIII animals were treated with 160 mg/lt concentration of lead acetate ,10% alcohol and Vitamin E/kg diet (AL-Jobory STA, 2006; Alkatan M, 2006).

1.2. Reagents used: 8% SDS: 8.0 gm of SDS (Sodium dodecyl sulfate) dissolved in 100 ml distilled water. 20% glacial acetic acid: 20 ml of glacial acetic acid made up to 100 ml with distilled water.0.8% TBA: 0.8gm of TBA (Thiobarbituaric acid) dissolved in 100 ml distilled water.Butanol:pyridine mixture (15:1): 1.0ml pyridine in 14.0 ml of n-butanol. Standard malondialdehyde (MDA): A 45 mM solution was prepared from 1,1,3,3-Tetra ethoxy propane obtained commercially. 1.0 ml of stock solution was diluted to 100 ml gives a concentration of 45 nmol of MDA/ml.

1.3. Lipid peroxidation Assay: The lipid peroxidation mixture contained 0.5 ml brain homogenate, 0.2 ml SDS, 1.5 ml acetic acid reagents, 1.5 ml TBA, and 0.7 ml distilled water. The tubes were placed in a boiling water bath for 1 hour. The samples were allowed to cool at room temperature. One ml of distilled water and a solution of 5.0ml butanol:pyridine was added (15:1) and mixed well. The tubes were centrifuged at 1000 rpm for 10 minutes. The colored layer was measured at 532 nm. The standards (1,1,3,3-tetra ethoxy propane) were processed as above and were read against water blank. Lipid peroxidation was expressed as nanomoles of thiobarbituric acid reactive substances formed/gm of wet brain (Okhawa H et al. 1979).

RESULTS

Lipid peroxidation at two weeks: In lead treated rats, the MDA value was 42.12nmol/gram and in alcohol treated rats, it was recorded 50.16nmol/gram of brain tissue. In lead with alcohol treated rats 67.15nmol/gram of MDA value was observed compared to control 25.20nmol/gram of brain tissue. In vitamin E treated rats, the MDA value was decreased and recorded 22.42nmol/gram of liver tissue. In lead with vitamin E treated rats, 40.12nmol/gram of MDA value was recorded and in alcohol with vitamin E treated rats, the MDA value was 49.21nmol/gram of liver tissue. In lead with alcohol and vitamin E treated rats, the MDA value was 70.17nmol/gram of brain tissue (Table 1 and Figure 1).

Table 1: ***Lipid peroxidation in rats treated for two weeks with lead, alcohol and lead combined alcohol with and without vitamin E treatment***

Group	Lipid peroxidation Mean ± SD
Control	$25.20^{b} \pm 0.0$
Lead	$42.12^{d} \pm 0.1$
Alcohol	$50.16^{f} \pm 0.0$
Lead + Alcohol	$67.15^{g} \pm 0.1$
Control + Vitamin E	$22.42^{a} \pm 0.2$
Lead + Vitamin E	$40.12^{c} \pm 0.0$
Alcohol + Vitamin E	$49.21^{e} \pm 0.0$
Alcohol + Lead + Vitamin E	$70.17^{h} \pm 0.0$

- ***Values are the mean of three replicates***
- ***± standard error.***
- ***The means followed by the same letter (S) are not significantly different at P<0.05 when subjected to Tukey's HSD.***

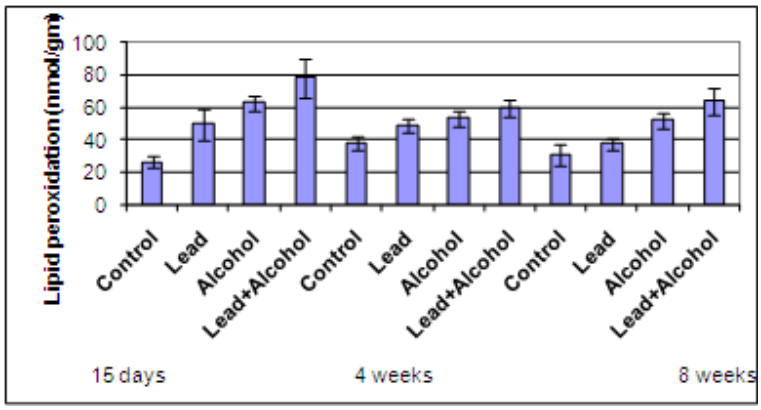

Figure1: ***Lipid peroxidation in rats treated for two weeks, four weeks and eight weeks with lead, alcohol and lead combined alcohol without vitamin E treatment***

Lipid peroxidation at four weeks: The lipid peroxidattion tested at four weeks of treatment recorded 52.82nmol/gram of brain tissue in lead treatment. In alcohol treated rats, the MDA value was 63.36nmol/gram. In lead and alcohol treated rats, the MDA value was 85.80nmol/gram. Compared to control, it was recorded 35.40 nmol/gram. In vitamin E treated rats, the MDA value was 51.26nmol/gram of brain tissue. In alcohol with vitamin E treated rats, the MDA value was 63.80nmol/gram and in alcohol, lead and vitamin E treated rats, the MDA value was 83.16nmol/gram of brain tissue(Table 2 and Figure 1).

Table 2: ***Lipid peroxidation in rats treated for four weeks with lead, alcohol and lead combined alcohol with and without vitamin E treatment***

Group	Lipid peroxidation Mean ± SD
Control	$35.40^{a} \pm 0.0$
Lead	$52.82^{d} \pm 0.1$
Alcohol	$63.36^{e} \pm 0.0$
Lead + Alcohol	$85.80^{h} \pm 0.1$

Control + Vitamin E	$37.62^{b} \pm 0.2$
Lead + Vitamin E	$51.26^{c} \pm 0.0$
Alcohol + Vitamin E	$63.80^{f} \pm 0.0$
Alcohol + Lead + Vitamin E	$83.16^{g} \pm 0.0$

- *Values are the mean of three replicates*
- *± standard error.*
- *The means followed by the same letter (S) are not significantly different at P<0.05 when subjected to Tukey's HSD.*

Table 3: ***Lipid peroxidation in rats treated for eight weeks with lead, alcohol and lead combined alcohol with and without vitamin E treatment.***

Group	Lipid peroxidation Mean ± SD
Control	$30.80^{c} \pm 0.0$
Lead	$58.74^{f} \pm 0.1$
Alcohol	$69.74^{g} \pm 0.0$
Lead + Alcohol	$86.68^{h} \pm 0.2$
Control + Vitamin E	$21.32^{a} \pm 0.0$
Lead + Vitamin E	$28.08^{b} \pm 0.0$
Alcohol + Vitamin E	$38.06^{d} \pm 0.0$
Alcohol + Lead + Vitamin E	$39.60^{e} \pm 0.0$

- ***Values are the mean of three replicates***
- ***± standard error.***
- ***The means followed by the same letter (S) are not significantly different at P<0.05 when subjected to Tukey's HSD.***

Lipid peroxidation at eight weeks: The data on the changes in MDA levels at eight weeks are presented in **Table 3** The percent change in MDA levels are presented in **Figure 1.** The MDA levels ranged from 25.52 to 42.24 nmol/gram tissue in the cerebral cortex of control rats (mean ± SD, 31.09 ± 6.96). A significant increase in MDA levels (mean ± SD, 37.71 ± 3.74), was seen after lead treatment. In lead treated rats, the MDA levels ranged from 35.20 to 44.88nmol/gram, and the percentage increase in MDA levels was 24 %. The MDA levels ranged from 44.00 to 59.84 nmol/gram in alcohol treated rats and the percentage increase in MDA levels was 68 %. In rats coexposed to lead and alcohol, the increase in MDA levels (mean ± SD, 63.89 ± 8.73) was more marked (110 %) compared to rats treated with alcohol or lead alone.

DISCUSSION

Lead is an environmental and occupational pollutant and it represents one of the major concern of public health. Lead adversely affects the nervous system resulting in mental retardation, impaired cognitive function and behavioral abnormalities. Alcohol abuse is associated with deleterious effects on several organs in the body including brain. Recently alcohol has been reported to enhance neurotoxic effects of lead. Intake of alcohol drinks is known to increase the blood level of lead. The average blood lead level in the alcoholics is almost twice as high as in people who are normal drinkers and can be higher than in those professionally exposed. As the production and consumption of alcohol as well as environmental lead pollution are increasing rapidly, there is increased incidence of coexposure of industrial workers as well as general population to alcohol and lead. However, there are few studies on the mechanism and mediators of alcohol and lead interactive neurotoxicity (Omodeo-Sale F et al. 1997).

The present study was aimed to examine short term and long term effects of lead and alcohol coexposure on oxidative damage and antioxidant defense in rat brain and effects of vitamin E administration on alcohol lead interactive neurotoxic effects. Although lipid peroxidation was more marked at two weeks of treatment compared to four weeks and eight weeks, the magnitude of oxidative stress

was still significant at four weeks and eight weeks. These results indicate ongoing oxidative stress during lead and alcohol treatment. The variations between different periods of time suggest adaptation to initial insult of toxicant. The lipid peroxidation caused by lead alone was significant but magnitude of increase in lipid peroxidation by lead was lower compared to administration of alcohol. Although potentiation of lead induced lipid peroxidation was not marked during coexposure to alcohol and lead, the effects of combined exposure to alcohol and lead were more than additive compared to either lead alone treated rats or alcohol alone treated rats. Oxidative stress induced by lead or alcohol is caused by either enhancing the production of oxygen reactive species and/or by decreasing the level of endogenous antioxidants.

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REFERENCE

1. Djebli N, Slimani M and Aoues A. Effect of lead exposure on Dopaminergic transmission in Rat brain. The International Journal of Child Neuropsychiatry 2004; 1 (1):363-368.
2. Flora J, Pande M, Kannan M and Mhta A. Lead induce oxidative stress and its recovery following co-adminstration of melatonin or nacetylcystiene during chelation with succimer in male rats. Cell Mol Biol.2004;50:543-551.
3. Goyer RA. Mechanism of lead and cadmium nephrotoxicity.Toxicol Lett.1989;46;153-162.
4. Fischbein A. Occupational and environmental lead exposure. In :Environmental and occupational medicine. Rom WN.(ed).2nd ed, Boston Little, Brown 1999;735-758.
5. Gerhardsson L, Endlyst V, Lndlyt V and Lundstrom N. Lead in tissues of decrease lead smelter workers. J Trace Elem Med Biol. 1995;9:236- 143.
6. Astrup JAS. Chemical contamination in human breast milk. Boston Rouge, LA:CRC Press 1991.
7. Lyn PND . Lead Toxicity Part II: The Role of Free Radical Damage and the Use of Antioxidants in the Pathology and Treatment of Lead Toxicity Lyn Patrick, ND Alternative Medicine Review.2006; 11(2) :114-127.
8. Ercal N, Neal R , Treeratphan P, Lutz PM, Hammond TC, Dennery PA and Spitz DR. A role for oxidative stress in suppressing serum immunoglobulin levels in lead exposed Fisher 344 rats. Arch. Environ. Contam. Toxicol. 2000; 39: 251-256.
9. Halliwell B, Gutteridge JMC. Protection against oxidants in biological systems: the superoxide theory of oxygen toxicity. In: Halliwell, B., Gutteridge JMC. Free radicals in biology and medicine, 2nd ed. Oxford: Clarendon Press. 1989: 86-123.
10. Okhawa H, Ohishi N and Yagi K. Assay for lipid peroxidation in animal tissues by thiobarbituric acid reaction. Anal Biochem. 1979; 95: 351-358.
11. Omodeo-Sale F, Gramigna D and Campaniello R. Lipid peroxidation and antioxidant systems in rat brain: effect of chronic alcohol consumption. Neurochem Res. 1997; 22: 577-82.
12. AL-Jobory STA. Reproductive efficiency of sucking rats treated with lead acetate during lactation :Role of vitamin E. MSc. Thesis college of Veterinary Medicine, University of Mosul, 2006 :22
13. Alkatan M. Effect of using some antioxidants on production performance and some physiological character in laying hens. PhD Dissertation, College of Agriculture and forestry, University of Mosul, 2006: 24-25.