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Research Article

STUDIES ON LIPID PEROXIDATION INDUCED BY LEAD, ALCOHOL ON LIVER AND AMELIORATION BY VITAMIN E

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ABSTRACT

Lipid peroxidation assay was carried in eight groups of animals treated with lead, alcohol and vitamin E individually and in combination for two weeks, four weeks and eight weeks respectively. In two weeks of treatment, lead recorded the Malondialdehyde (MDA) value of 8.11 nmol/gram liver tissue, alcohol (9.57 nmol/gram liver tissue), lead acetate with alcohol recorded 11.29 nmol/gram liver tissue. Compared to control, it was recorded 7.08 nmol/gram liver tissue. When treated with vitamin E, the MDA value was decreased in all the combination of treatment. In four weeks of treatment, maximum lipid peroxidation activity was observed in lead with alcohol treatment (17.11 nmol/gram liver tissue) and least was observed in lead (11.41 nmol/gram liver tissue). The lipid peroxidation activity was decreased in vitamin treatment along with lead alcohol treatment. In eight weeks of treatment, maximum lipid peroxidation activity was observed in lead with alcohol and recorded 17.99 nmol/gram liver tissues. Compared to control, it was recorded 8.79 nmol/gram liver tissue. In vitamin E with lead and alcohol treated animals, it was recorded 13.83 nmol/gram liver tissue. Compared to control with only vitamin E, it was recorded 8.53 nmol/gram liver tissue.

Key Words: Lipid peroxidation, Malondialdehyde (MDA), lead, Alcohol, Vitamin E, Liver tissue.

INTRODUCTION

Lead (Pb) exposure/toxicity is a leading environmental health issue for children and women of childbearing age.[1] Lead is an environmental toxicant. The liver, kidneys and brain have been considered as the target organs for the toxic effects of lead. Many recent reports showed that Pb exposure continues to be a major public health problem worldwide[2, 3]. Animal studies have confirmed that exposure to low levels of Pb during the perinatal period can produce long-lasting changes in the brain[4].Lead is an ubiquitous element in the environment, it is used in many industrial activities including mining, refining and producing lead – acid batteries[5]. Animals may be exposed to lead via contaminated food or water and fuel additives.[6] The alimentary and respiratory tract are the major routes of lead entry into the body.[7] Once the lead is in the bloodstream, it is distributed into soft and hard tissues.[8] Alcohol abuse is associated with deleterious effects on several organs in the body, particularly liver and brain. Alcoholics have been found to be more prone to lead intoxication. Higher blood lead levels have been reported in alcoholic industrial workers exposed to lead compared to non-alcoholics indicating the alcoholics might be more prone to the toxic effects of this metal than non alcoholics. As the production and consumption of ethanol as well as environmental lead pollution are increasing rapidly, there is every likelihood of co exposure of industrial workers as well as general population. However, few reports are available on the effects of ethanol on lead induced biochemical changes. Although several mechanisms have been proposed to explain the lead-induced toxicity, no mechanisms have been yet defined clearly. Experimental studies concerning the effects of lead exposure perinatally on social behavioral of the offspring during age are very limited. Recent studies suggest oxidative stress as one of the important mechanisms of toxic effects of lead. The results of ethanol treatment on lipid peroxidation and oxidative stress are conflicting. The changes in parameters were found to be increased, normal and decreased. Fewer reports are available on combined treatment of alcohol and lead on hepatotoxicity and oxidative stress. In the present study, the effect of lead, alcohol and vitamin E on lipid peroxidation and oxidative stress on liver tissue were studied.

MATERIALS AND METHODS

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.

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. [9, 10, 11]

Chemicals Used

8.0% SDS (8.0 grams of SDS (Sodium Dodecyl Sulphate) dissolved in 100ml distilled water). 20% glacial acetic acid (20ml of glacial acetic acid make up to 100 ml with distilled water). 0.8% TBA(0.8 grams of TBA (Thiobarbituric Acid) dissolved in 100ml distilled water). Butanol pyridine mixture (15:1) (1.0ml pyridine in 14.0ml of n-butanol). Standard malondialdehyde (MDA)(A 45mM solution was prepared from 1,1,3,3- Tetra ethoxy propane obtained commercially. 1.0ml of stock solution was diluted to 100ml gives a concentration of 45nmole of MDA/ml).

Lipid Peroxidation Assay

The quantitative determination of lipid peroxidation was assessed by thiobarbituric acid reactive species and malondialdehyde concentration was calculated. Determination of the lipid peroxide content of blood and tissue sample is performed indirectly by means of derivatising Malondialdehyde (MDA) with Thiobarbituric acid (TBA) at high temperature and acidic conditions. Thiobarbituric acid reacts with MDA to form a diadduct, a pink chromogen, which was measured spectrophotometrically at 532 nm[12].

The lipid peroxidation mixture contains 0.5 ml liver homogenate, 0.2 ml SDS, 1.5 ml acetic acid reagent, 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, distilled water (1.0 ml) 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 532nm.The standards (1, 1, 3, 3, -tetra ethoxypropane) were processed as above and was read against water blank. Lipid peroxidation was expressed as nanomoles of thiobarbituric acid reactive substances formed per gram of wet liver[12] .The lipid peroxidation activity was carried at two weeks, four weeks and eight weeks respectively. The Malondialdehyde (MDA) accumulation determined as thiobarbituric acid reactive substances was used as an indicator of oxidative stress and lipid peroxidation.

RESULT

Lipid peroxidation at Two weeks

Among the eight groups of animals treated, the MDA value recorded 8.11nmol/gram in lead treated animals. Alcohol treated animal recorded 9.57nmol/gram followed by lead alcohol which was recorded 11.29nmol/gram of liver tissue. Compared to control , it was recorded 7.08nmol/gram of tissue. In vitamin E treated animals, lead with vitamin treated animals recorded 6.02nmol/gram. Alcohol with vitamin E recorded 8.72 nmol/gram and lead with alcohol and vitamin E recorded 9.81 nmol/gram of tissue. Compared to control with Vitamin E, the MDA value recorded 4.11nmol/gram (Table 1).

TABLE 1: Lipid Peroxidation Activity in Rats Treated For Two Weeks, Four Weeks and Eight Weeks, With Lead, Alcohol And Alcohol And Lead Without Vitamin E Treatment.

Group	Lipid peroxidation (nmol/gram)
Control	7.08^{c}±0.0
Lead	8.11^{d} ±0.1
Alcohol	9.57^{f}±0.1
Lead + Alcohol	11.29^{h}±0.0
Control + Vitamin E	4.11^{a}±0.0
Lead + Vitamin E	6.02^{b}±0.1
Alcohol + Vitamin E	8.72^{e}±0.2
Lead + Alcohol + Vitamin E	9.81^{g}±0.1

- **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 Four weeks

Animals when treated for four weeks, lead treated animals recorded MDA of 11.41nmol/gram. Alcohol treated animals recorded 12.63nmol/gram. Lead with alcohol treated animals recorded 17.11 nmol/gram. Compared to control, it was recorded 8.44 nmol/gram respectively. 9.36 nmol/gram of MDA value was recorded in lead with vitamin E treated animals and 10.94 nmol/gram was recorded in alcohol with vitamin E treated animals and 12.46 nmol/gram was recorded in lead with alcohol and vitamin E treated animals. Compared to control with vitamin E, it was recorded 6.42 nmol/gram of tissue (Table 2).

Lipid peroxidation at Eight weeks

In eight weeks of treatment, lead treated animals recorded MDA value of 11.23 nmol/gram of tissue. Alcohol trated animals recorded 14.03 nmol/gram. In lead with alcohol treated animals MDA value was recorded 14.03 nmol/gram of tissue. Compared to control, it was recorded 8.79 nmol/gram. In Vitamin E with lead treated animals, it was recorded 10.28 nmol/gram followed by alcohol with vitamin E and recorded 11.77 nmol/gram. In lead alcohol with vitamin E treated animals, it was recorded 13.83 nmol/gram of tissue. Compared to control with vitamin E recorded 8.53 nmol/gram of tissue (Table 3).

TABLE 2: Lipid Peroxidation Activity in Rats Treated For Four Weeks With Lead, Alcohol And Alcohol And Lead With And Without Vitamin E.

Group	Lipid peroxidation (nmol/gram)
Control	8.44^{b}±0.1
Lead	11.41^{e}±0.0
Alcohol	12.63^{g}±0.1
Lead + Alcohol	17.11^{h}±0.0
Control + Vitamin E	6.42^{a}±0.0
Lead + Vitamin E	9.36^{c}±0.1
Alcohol + Vitamin E	10.94^{d}±0.0
Lead + Alcohol + Vitamin E	12.46^{f}±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 Activity in Rats Treated For Eight Weeks With Lead, Alcohol And Alcohol And Lead With And Without Vitamin E.

Group	Lipid peroxidation (nmol/gram)
Control	8.79^{b}±0.0
Lead	11.23^{d}±0.1
Alcohol	14.03^{g}±0.0
Lead + Alcohol	17.99^{h}±0.0
Control + Vitamin E	8.53^{a}±0.1
Lead + Vitamin E	10.28^{c}±0.1
Alcohol + Vitamin E	11.77^{e}±0.0
Lead + Alcohol + Vitamin E	13.83^{f}±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.**

DISCUSSION

Lead is a common environmental and industrial pollutant that has been detected in all phases of environment and biological system. The persistence of lead in the animals and humans and the associated health risk is a topic of current debate and concern.[13] Lead has been found to produce wide range of toxic-biochemical effects, besides behavioral dysfunction in man and in experimental animals.[14] Liver, kidneys and brain have been considered as the target organs for the toxic effects of lead. Chronic exposure to this biotoxicant leads to its accumulation in these organs with maximum concentration per gram weight of tissue being recorded in kidneys.[15] The hepatotoxic effects of lead include potentiation of endotoxin mediated liver injury and apoptosis of hepatocytes.[16]

Alcoholic liver disease is a major health problem worldwide. In addition, alcohol has been shown to potentiate lead induced cytotoxic effects. Although mechanism of alcohol potentiation of lead toxicity is not clearly known, alcohol induced excessive accumulation of lead is one of the potential important mechanism by which alcohol increased lead induced toxic effects. The accumulation of lead in alcoholics under the normal level of lead in the

environment often exceeds the level of lead in tissues of individuals who are at risk for exposure to lead such as persons working with lead related products.[17]

Lead-alcohol interactive toxicity has been emerged as an important public health concern because of increase consumption of alcohol by individuals who are increasingly exposed to lead contamination. However, the mechanism and mediators of lead-alcohol interactive toxicity are not known.

Alcohol potentiation of lead induced oxidative stress Oxidative stress has been implicated to contribute to lead-associated tissue injury in the liver, kidneys, brain and other organs.[18] Indirect in vivo evidence of oxidative involvement in lead-induced pathotoxicity was demonstrated by alleviation of oxidative stress in the erythrocytes after treatment with thiol containing proven antioxidants such as, N-acetyl cysteine, in lead exposed rats.[19] Also reactive oxygen species related to lead toxicity in the rat sperm was prevented by supplementation of rat fed with vitamin E and or vitamin C.[20]

The mechanism for lead-induced oxidative stress include the effect of lead on membrane and antioxidant defense system of cells. On cell membrane, the presence of double bonds in the fatty acids weakens the C-H bonds on the carbon atom adjacent to the double bonds and makes hydrogen atom removal easier. Another mechanism for lead induced membrane oxidative damage is the effect on changes in the fatty acid composition of membrane. Lead induced arachidonic acid elongation might be responsible for the enhanced lipid peroxidation in the membrane. By causing lateral phase separation and/or by increasing lipid peroxidation rates, lead could affect membrane-related processes such as the activity of membrane enzymes, endo and exocytosis, the transport of solutes across the bilayer, and signal transduction process.[18]

The δ-aminolevulinic acid dehydratase is highly sensitive to the toxic effects of lead .The accumulation of δ-aminolevulinic acid induces generation of reactive oxygen species.[21] and oxidative stress.[22]

Another mechanism for lead-induced oxidative stress is on the antioxidant defense system of cells. Lead has a high affinity for sulfhydryl (SH) groups and mercaptides are formed with the sulfhydryl group of cysteine, and less stable complex with other amino acid side chains . Lead is shown to alter antioxidant activities by inhibiting functional sulfhydryl groups in several enzymes such as δ-aminolevulinic acid dehydratase, superoxide dismutase, catalase, glutathione peroxidase and glucose-6 phosphate dehydrogenase.[23]

Several mechanism responsible for alcohol-induced hepatotoxicity, include oxidative stress and lipid peroxidation, immunogenic process initiated by formation of protein adducts of acetaldehyde other aldehydes and I-hydroxyethyl radicals; and activation of kupffer cells by endotoxin and subsequent cascade of events that involved cytokines, chemokines, and adhesion molecules. Increasing evidence implicates enhanced intestinal permeability caused by alcohol ingestion is responsible for endotoxemia.

Although there is considerable evidence from both human and animal studies that alcohol consumption enhances oxidative stress in liver and other tissues, the results are conflicting and opinion differ as to the role of oxidative stress and its relevance for hepatotoxicity, after alcohol exposure. Lipid peroxidation in the liver has been shown after acute as well as chronic alcohol administration ,but few reports indicate absence of oxidative stress during acute and chronic exposure to ethanol. Although alcohol has been shown to potentiate lead induced oxidative stress in the liver, the time dependent effects have not been investigated. In the present study, the results indicate the presence of oxidative stress when examined at 2 weeks, 4 weeks and 8 weeks. Moreover the potentiating effects are seen both during acute and chronic exposure to lead and alcohol.

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