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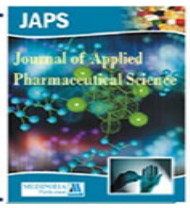

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Evaluation of Effects of Different Concentrations of Lead, Alcohol and Vitamin E on Protein Carbonyl Content of Brain Tissue in Rats

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ABSTRACT

The lead treatment was characterized by increase (28%) in protein carbonyl content 5.19, but the increase was not significant. In alcohol treated rats, the protein content was 5.52nmol/gram of brain tissue of rat. The increase (36%) was significantly higher when compared to lead treated rats. The protein carbonyl content (6.39nmol/gram) was further increased in rats coexposed to alcohol and lead, and the values ranged from 5.40 to 8.20 nmol/gram. The percent increase in protein carbonyl content was 57% in rats coexposed to alcohol and lead. Thus, the magnitude of deleterious effects of oxidative stress on protein oxidation is more significant in combined treatment group when compared to lead alone treated rats and alcohol alone treated rats. The lead treatment was characterized by a significant increase (37%) in protein carbonyl content (6.19nmol/gram). In alcohol treated rats, the increase in protein carbonyl content (6.79nmol/gram) was more marked (51%) when compared to lead treated rats. The protein carbonyl content was significantly increased (73%) in rats coexposed to alcohol and lead, and the values ranged from 6.80 to 8.56 nmol/gram (7.76nmol/gram).

Keywords: Lead, Alcohol, Vitamin E, Protein, Brain tissue.

INTRODUCTION

Lead (Pb) is a ubiquitous heavy metal. Its exposure mainly occurs through the respiratory and gastrointestinal systems. Absorbed Pb (whether inhaled or ingested) is stored in soft tissues. Autopsy studies of Pb-exposed humans indicate that liver tissue is the largest repository (33%) of Pb among the soft tissues followed by kidney cortex and medulla (Veena *et al*., 2011). Effects of lead on nervous system depend on the duration of exposure and its intensity, which could be assessed by concentrations of lead in the blood. Children are particularly susceptible to toxic lead effects on central nervous systems. Elevated blood lead level impairs both physical and intellectual development in children. Effects of high blood lead concentrations are widely discussed (Kmiecik-Małecka *et al*., 2009).

Low-level exposures to lead, resulting in blood lead levels previously considered normal, may cause cognitive dysfunction, neurobehavioral disorders, neurological damage, hypertension, and renal impairment. The pathogenesis of lead toxicity is multifactorial, as lead directly interrupts enzyme activation, competitively inhibits trace mineral absorption, binds to sulfhydryl proteins (interrupting structural protein synthesis), alters calcium homeostasis, and lowers the level of available sulfhydryl antioxidant reserves in the body (Lyn, 2006). Lead-induced oxidative stress contributes to the pathogenesis of lead poisoning for disrupting the delicate prooxidant/antioxidant balance that exists within mammalian cells. Production of reactive oxygen species (ROS) is increased after lead treatment in vitro studies, moreover other studies in vivo suggest that lead exposure cause generation of ROS and alteration of antioxidants defense system in animals (Hassan and Jassim, 2010). In the present investigation, lead, alcohol and Vitamin E was feed to rat individually and in combination and its effect its effect on protein content in brain tissue of rat was analyzed.

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 (AL-Jobory STA, 2006; Alkatan M, 2006).

Chemicals used

Alkaline copper reagent Solution A (2% sodium carbonate in 0.1 N NaOH solution in distilled water). Solution B (0.5% copper sulphate in 1.0% sodium potassium tartarate). 50.0 ml of solution A was mixed with 1.0ml of solution B just before use. Folin's phenol reagent (The reagent was obtained commercially). One volume of Folin's phenol reagent was diluted with 1 volume of distilled water just before use. Standard bovine serum albumin (BSA) (20.00 mg of BSA was dissolved in 100 ml distilled water).

Few drops of NaOH was added to aid complete dissolution of BSA and to avoid frothing. It was allowed to stand overnight. 10ml of the stock was diluted to 100ml to get a working standard (200 µg/ml).

Estimation of total protein carbonyl content

The protein content was determined by Folins phenol (Lowry *et al.,* 1951) method using bovine serum albumin as standard. 0.1ml crude homogenate was used for protein extraction. The protein was extracted by mixing with 5% cold trichloroacetic acid and centrifuged. The pellet was solubilized with 0.5 N sodium hydroxide and stored overnight at room temperature. After neutralization with 0.5 N HCl, 0.2 ml of diluted solution and different concentrations of standard were taken. The volume was made up to 1.0 ml with distilled water. Blank contained 1.0 ml distilled water. To all the tubes 5.0 ml alkaline copper reagent was added and left at room temperature for 10 Min. 0.5 ml of folin's phenol reagent was added and the blue color developed was read after 20 minutes at 660nm against reagent blank in a spectrophotometer. Protein concentration is expressed as mg/gm of wet brain tissue.

RESULT

Protein carbonyl content for four weeks

In four hours of treatment, lead treated rats recorded 5.19 nmol/gram of protein in brain tissue of rats. In alcohol treated rats, the protein carbonyl content was 5.52nmol/gram and in lead combined with alcohol treatment, it was recorded 6.39 nmol/gram. Compared to control, it was recorded 4.07 nmol/gram of tissue. When treated with vitamin E, the protein carbonyl content was 4.06nmol/gram and in lead with vitamin E treated rats, it was recorded 4.85 nmol/gram. In alcohol with vitamin E treated rats, it was recorded 5.14 and in lead with alcohol and vitamin E treatment, the protein carbonyl content was 5.68nmol/gram of brain tissue (Table 1 and Figure 1).

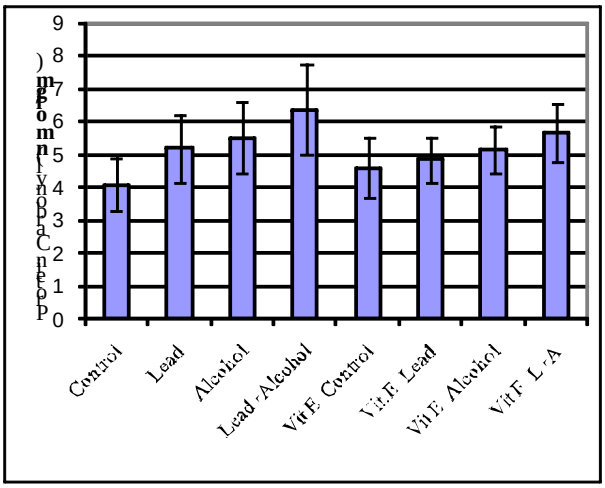

Fig. 1: Protein carbonyl content in rats treated for four weeks with lead, alcohol and lead with alcohol, with and without vitamin E.

Table. 1: Protein carbonyl content in rats treated for four weeks with lead, alcohol and lead with alcohol ,with and without vitamin E treatment.

Group	Protein carbonyl content Mean ± SD
Control	$4.07^{a} \pm 0.0$
Lead	$5.19^{e} \pm 0.0$
Alcohol	$5.52^{f} \pm 0.1$
Lead + Alcohol	$6.39^{h} \pm 0.1$
Control + Vitamin E	$4.60^{b} \pm 0.0$ (13.02%↑)**
Lead + Vitamin E	$4.85^{c} \pm 0.0$ (6.56%↓)
Alcohol + Vitamin E	$5.14^{d} \pm 0.0$ (6.89%↓)
Alcohol + Lead + Vitamin E	$5.68^{g} \pm 0.1$ (11.11%↓)

* Values were expressed as nmol/gm. a = significant at p<0.05 vs. control, b = significant at p<0.05 vs. lead. c = significant at p<0.05 vs. alcohol. ** The values in the parenthesis indicate percent change from the corresponding group without vitamin E.

Protein carbonyl content for eight weeks

In eight weeks of treatment, the protein carbonyl content was 6.19nmol/gram in lead treated rats. In alcohol treated rats, the protein carbonyl content was 6.79 and in lead with alcohol treated rats, the protein carbonyl content was 7.76nmol/gram of brain tissue. Compared to control, it was recorded 4.57nmol/gram. In vitamin E treated rats, the protein content was 4.18 and in lead with vitamin E treated rats, the protein content was 4.68nmol/gram of tissue. In alcohol with vitamin E treated rats, it was recorded 5.02 and in alcohol with vitamin E and lead treated rats, the protein carbonyl content was 6.09nmol/gram of brain tissue compared to control (Table 1 and Figure 2).

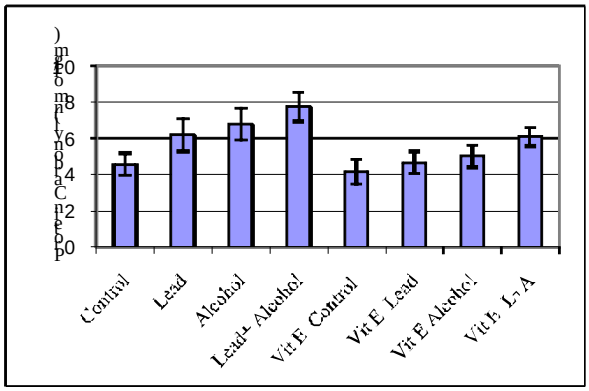

Fig. 2: Protein carbonyl content in rats treated for eight weeks with lead, alcohol and lead with alcohol, with and without vitamin E treatment.

Table. 2 : Protein carbonyl content in rats treated for eight weeks with lead, alcohol and lead with alcohol with and without vitamin E treatment.

Group	Protein carbonyl content Mean ± SD
Control	$4.57^{b} \pm 0.1$
Lead	$6.19^{f} \pm 0.1$
Alcohol	$6.79^{g} \pm 0.0$
Lead + Alcohol	$7.76^{h} \pm 0.0$
Control + Vitamin E	$4.18^{a} \pm 0.0$ (8.5%↓)**
Lead + Vitamin E	$4.68^{c} \pm 0.0$ (24.4%↓)
Alcohol + Vitamin E	$5.02^{d} \pm 0.1$ (26.07%↓)
Alcohol + Lead + Vitamin E	$6.09^{e} \pm 0.1$ (21.5%↓)*

* Values were expressed as nmol/gm. a = significant at p<0.05 vs. control, b = significant at p<0.05 vs. lead. c = significant at p<0.05 vs. alcohol.. ** The values in the parenthesis indicate percent change from the corresponding group without vitamin E.

DISCUSSION

A reduction in the rate of protein synthesis is a central feature of acute alcohol toxicity affecting diverse range of tissues such as the salivary glands, gastrointestinal tract, skeletal muscle, heart and brain (Bonner *et al.*, 2000). These reductions are reported to be related to changes in the activation of translation initiation factors involved in the binding of met-tRNA to the 4OS ribosomal unit i.e., eukaryotic initiation factor 2B (elF2B) and the initiation factors that are involved in the binding of mRNA to 43S preinitiation complex elF4E (Lange *et al.*, 2001). Muscle and liver respond differently with respect to these subcellular changes (Lange *et al.*, 2000). In skeletal muscle, reductions in protein synthesis may be an initiating factor in the pathogenesis of the disease entity alcoholic myopathy (Reilly *et al.*, 1995). However, cellular factors for initiating the reductions in tissue protein synthesis in alcohol toxicity are unknown, although consideration must be given to the excessive generation of reactive oxygen species (ROS), leading to enhanced lipid peroxidation and/or membrane damage (Adachi *etal*., 2000). Two cholesterol-derived hydroperoxides, 7α-hydroxyperoxycholest-5-en-3β-ol (7α-OOH) and 7β-hydroxyperoxycholest-5-en-3β-ol (7β-OOH), were significantly elevated in both soleus and plantaris muscle of rats. Numerous studies have also implicated the generation of ROS and/or enhanced lipid peroxidation in the pathogenesis of reduced tissue protein synthesis. The shellfish toxin okadaic acid reduces protein synthesis in cultures of vero cells, by a mechanism involving increased lipid peroxidation (Matias *et al.*, 1999). In metabolically degenerated neuronal tissue in vitro and liver tissue slices, there were also decreased in protein synthesis in response to enhanced lipid peroxidation (Uto *et al.*, 1995). In the present study, the protein carbonyl content was higher in eight weeks of treatment compared to four weeks of treatment. In eight weeks of treatment, there is increase in protein content in lead(1.02%), alcohol(1.10%) and in lead with alcohol treatment, the percentage of increase in upto 1.38% . From the present study, it can be concluded that as the duration of treatment increases the protein carbonyl content goes on increasing . A further work in necessary to determine the mode of action of lead, alcohol and vitamin E on the brain tissue of rats.

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