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ANALYSIS AND DETECTION OF EFFECT OF LEAD AND ALCOHOL ON VITAMIN C OF BRAIN TISSUE OF RATS

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ABSTRACT

In vitro evaluation of effect of lead and alcohol on vitamin C content on brain tissue was evaluated at four and eight weeks of duration in brain tissue of rats. In four hours of treatment, The vitamin C content ranged from 393.0 µg/gram in the cerebral cortex of control rats. The lead treatment was characterized by a decrease in vitamin C content 336.5µg/gram. In alcohol treated rats, the decrease in vitamin C content 233.0µg/gram were higher compared to lead treated rats. In eight hours of treatment, the brain tissue of rats recorded the vitamin C content was 315.0 µg/gram in the cerebral cortex of control rats. The lead treatment was characterized by a marginal decrease in vitamin C content 284.0µg/gram In alcohol treated rats, the decrease in vitamin C content 256.0 µg/gram. The vitamin C content were decreased in rats coexposed to alcohol and lead, and recorded 205 µg/gram.

Key words: Vitamin C, Lead, Alcohol, Vitamin E, Brain tissue, Rats.

INTRODUCTION Vitamin C is required for the growth and repair of tissues in all parts of your body. It is necessary to form collagen, an important protein used to make skin, scar tissue, tendons, ligaments, and blood vessels. Vitamin C is essential for the healing of wounds, and for the repair and maintenance of cartilage, bones, and teeth. Vitamin C is one of many antioxidants. Vitamin E and beta-carotene are two other well-known antioxidants. Antioxidants are nutrients that block some of the damage caused by free radicals, which are by-products that result when our bodies transform food into energy. Lead (Pb) is environmental and indiustrial pollutant that has undesired effects, including growth retardation, immunological, hepatic and reproductive disfunctions. Pb caused lymphocyte

infiltration and cirrhosis of liver cells. Hepatic disorders were noticed in rats as manifested by increase of liver enzymes. Activation of free radical processes and impairment of the antioxidant defense system are likely to be one of the basic mechanisms responsible for hepatic damage during prolonged lead intoxication . Treatment of rats with Pb increased reactive oxygen species (ROS) that may contribute to hypertension and tissue damage[1]. In the nervous system, all neurons and glial cells form a very large network, integrate all external and internal stimuli and contributes to the elaboration of adequate responses[2]. Alcohol consumption represents a large problem all over the world[3]. Alcohol cannot be stored and obligatory oxidation must take place predominantly in the liver via alcohol dehydrogenase. The production of potentially toxic acetaldehyde is enhanced and conversion to acetate reduced [4]. In the present study effect of lead and alcohol on vitamin C content on brain tissue of rats was tested.

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 [5,6].

Reagents used: TCA 10 %(10gm trichloro acetic acid make up to 100ml with distilled water). 2, 4-Dinitrophenylhydrazine (DNPH)(Thiourea-copper sulphate (DTC) reagent: 3.0 gms of DNPH, 0.4gm thiourea, and 0.05gm copper sulphate were dissolved in 100ml of 9N

H_2SO_4). Sulphuric acid 65% (v/v) (65.0 ml of concentrated H_2SO_4 was mixed with 35ml of water) and Standard vitamin C (50.0 mg of vitamin C was dissolved in 100ml of 4% TBA).

Vitamin C Assay

Vitamin C was estimated by the colorimetric method[7]. 0.5 ml of tissue homogenate , 0.5 ml of distilled water and 1.0 ml of 10% TCA were added, mixed thoroughly and centrifuged for 20 minutes. 1.0 ml of the supernatant and 0.2 ml of (DTC reagent) 2,4 dinitro phenylhydrazine–thiourea–copper sulphate reagent was added and incubated at 37°C for 3 hours. Then 1.5 ml of 65% sulphuric acid was added, mixed well and allowed to stand at room temperature for another 30 minutes. The color developed was read at 520 nm. Graded amount of standards were also treated similarly. Vitamin C level was expressed as µg/gm of wet brain.

RESULTS

Treatment of rats for four weeks

The data on vitamin C content in rats treated with lead, alcohol and coexposed to lead and alcohol at four weeks are given in Table1 and Figure 1. The vitamin C content ranged from 244 to 500 µg/gram tissue (mean ± SD, 393.0 ± 108.08) in the cerebral cortex of control rats. The lead treatment was characterized by a decrease in vitamin C content (mean ± SD, 336.5 ± 73.91). In lead treated rats, the vitamin C content ranged from 250 to 396 µg/gram tissue. The percentage decrease in vitamin C content was 13%, which in lead treated rats. In alcohol treated rats, the decrease in vitamin C content (mean ± SD, 233.0 ± 12.12) were higher (30%) compared to lead treated rats. The vitamin C content were also decreased in rats coexposed to alcohol and lead, and the values ranged from 214 to 230 µg/gram tissue (mean ± SD, 223.0 ± 6.81). The percent decrease in vitamin C content was 39% in rats coexposed to alcohol and lead. Thus, decrease in vitamin C content was more significant in alcohol-lead co exposed rats compared to rats treated with alcohol or lead alone.

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

Group	Vitamin C Mean ± SD
Control	393.0 ± 108.08
Lead	336.5 ± 73.91
Alcohol	233.0 ± 12.12
Lead + Alcohol	223.0 ± 6.81[ab]
Control + Vitamin E	460.5 ± 79.79 (17.17%↑)**
Lead + Vitamin E	382.5 ± 64.53 (13.67%↑)
Alcohol + Vitamin E	264.0 ± 14.0[ab] (13.30%↑)
Alcohol + Lead + Vitamin E	245.0 ± 26.81[ab] (9.87%↑)

* Values were expressed as microgram/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.

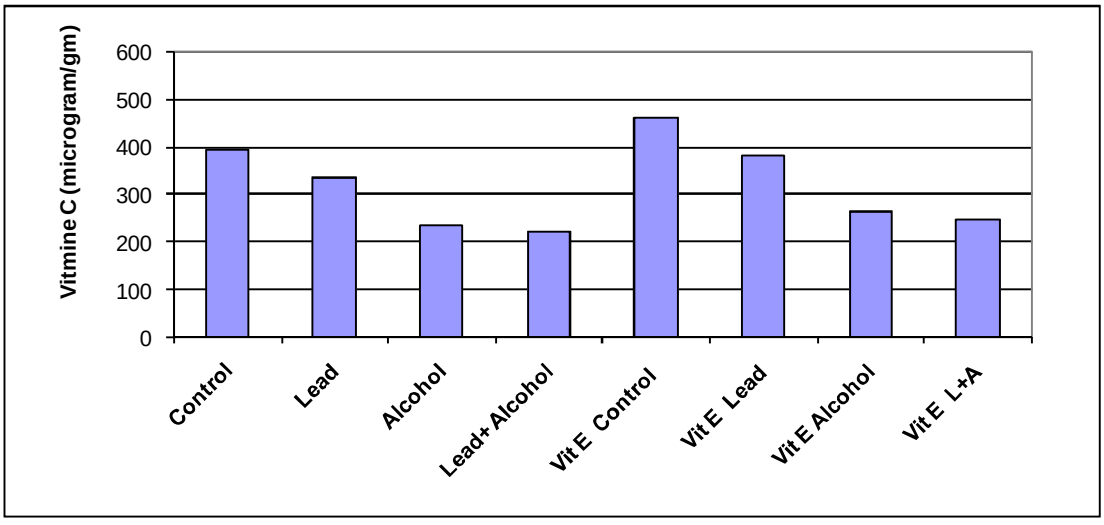

Figure1: Vitamin C content in rats treated for four weeks with lead, alcohol and lead with alcohol with and without vitamin E treatment.

Treatment of rats for Eight weeks Vitamin C content in rats treated with lead, rats treated with alcohol and rats coexposed to lead and alcohol for eight weeks are given in Table 2 and Figure 2. The vitamin C content ranged from 294 to 338 μg/ gram tissue (mean ± SD, 315.0 ± 18.29) in the cerebral cortex of control rats. The lead treatment was characterized by a marginal decrease (10%) in vitamin C content (mean ± SD, 284.0 ± 20.72). In alcohol treated rats, the decrease in vitamin C content (mean ± SD, 256.0 ± 36.26) was more significant (18%) compared to lead treated rats. The vitamin C content were significantly decreased (35%) in rats coexposed to alcohol and lead, and the values ranged from 140 to 278 μg/gram tissue (mean ± SD, 205 ± 56.53).

Table 2: Vitamin C content in rats treated for eight weeks with lead, alcohol and lead with alcohol with and without vitamin E treatment

Group	**Vitamin C** **Mean ± SD**
Control	315.0 ± 18.29
Lead	284.0 ± 20.72
Alcohol	256.0 ± 36.26 [a]
Lead + Alcohol	205.0 ± 56.53[ab]
Control + Vitamin E	330.00 ± 6.00 (4.76%↑)**
Lead + Vitamin E	318.67 ± 14.05 (12.2%↑)
Alcohol + Vitamin E	303.33 ± 21.19 (18.49%↑)
Alcohol + Lead + Vitamin E	274.00 ± 42.33 (33.66%↑)

* Values were expressed as microgram/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.

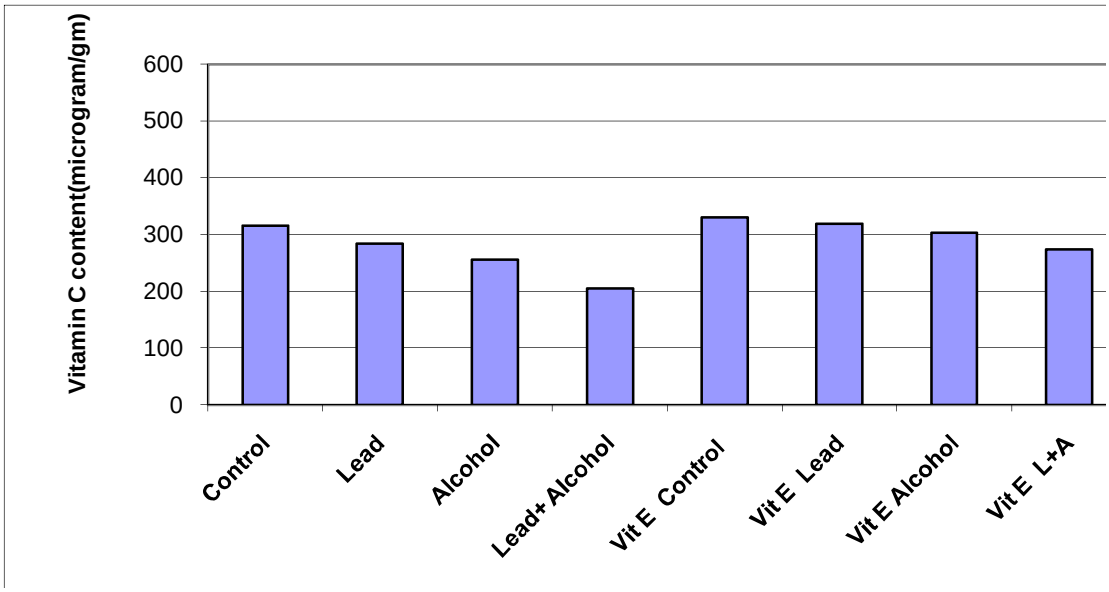

Figure 2. Vitamin C content in rats treated for eight weeks with lead, alcohol and lead with alcohol with and without vitamin E treatment.

DISCUSSION

Although increased catalase could account for increased oxidative stress due to metabolism of alcohol in the context of increased glutathione status and near normal vitamin E levels, oxidative stress occurring in lead treated animals are not accounted by these biochemical changes. The results of vitamin C content offer partial explanation for the ongoing stress in the presence of adaptive changes in catalase and glutathione system. Even at four weeks of treatment, the concentration of ascorbic acid was found to be decreased in lead and alcohol treated animals. The interaction between vitamin E and other antioxidants might have been a more efficient protective action against lead toxicity. Vitamin E and C jointly protect lipid structures against peroxidation[8]. Although vitamin E is located in membranes and vitamin C in aqueous phases, vitamin C is able to recycle oxidized vitamin E [9]. Vitamin C repairs the tocopherol radical, thus recovering the chain-breaking antioxidant capacity of vitamin E [8]. These results suggest that vitamin C is a predominant antioxidant system that is compromised during lead and alcohol exposure. Vitamin C, an important water-soluble free radical scavenging antioxidant is commonly reported to be reduced in chronic alcoholic patients. Recent studies during which the present study is in progress, support our findings. In rats administered alcohol and ascorbic acid, malondialdehyde (MDA), hydroperoxide and conjugated dienes decreased in comparison with that given alcohol alone[10].

CONCLUSION

From the above observation it can be concluded that, the lead and Alcohol have strong negative effect on vitamin C activity in brain tissue of rats. Hence the lead and alcohol in pure form and in combination have direct effect on vitamin C activity. But when Vitamin E was treated along with lead and alcohol individually and in combination the activity of Vitamin E was also reduced by Lead and Alcohol.

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