

## Estimation of Effect of Lead, Alcohol and Vitamin E on Aspartate Amino Transferase and Alanine Amino Transferase of Liver Tissue in Rats

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### ABSTRACT

Eight groups of rats, each group consists of six animals. Group I acts as control receiving water. Group II were treated with lead acetate at 160mg/ liter concentration dissolved in water. Group III animals were treated with 10% alcohol. Group IV animals were treated with 160 mg/ liter concentration of lead acetate and 10% alcohol. Group V animals served as control treated with 500mg Vitamin E/kg diet. Group VI animals were treated with lead acetate at 160 mg/ liter 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/ liter concentration of lead acetate , 10% alcohol and 500mg Vitamin E /kg diet. Effect of alcohol, lead and vitamin E for eight weeks on AST (Aspartate amino transferase) and ALT (Alanine amino transferase) of liver tissue was evaluated in *in vitro* condition. When treated with lead, the AST was recorded 92.50U/L, 100.0 U/L in alcohol, 119.67 U/L in lead(160mg/ liter) with alcohol(10%). The AST was 54.50U/L in vitamin E (500mg/kg diet) treated tissue. 76.75 U/L in lead and vitamin E treated tissue, 85.75U/L in alcohol, vitamin E treated tissue and 102.25U/L in lead, alcohol and vitamin E treated liver tissue. ALT recorded 50.50 U/L in lead treated tissue, 53.25 U/L in alcohol treated tissue, 67.75 U/L in lead with alcohol treatment, 32.24 U/L in vitamin treated liver tissue, 37.33 U/L in lead with vitamin E treated tissue, 51.75 U/L in alcohol and vitamin treated tissue and 57.0 U/L in lead, alcohol and vitamin E treated liver tissue.

**Keywords:** Alcohol, alanine amino transferase, aspartate amino transferase, lead, liver, vitamin E

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### INTRODUCTION

An aspartate aminotransferase (AST) test measures the amount of this enzyme in the blood. AST is normally found in red blood cells, liver, heart, muscle tissue, pancreas, and kidneys. AST formerly was called serum glutamate oxaloacetate transaminase (SGOT). Low levels of AST are normally found in the blood. When body tissue or an organ such as the heart or liver is diseased or damaged, additional AST is released into the bloodstream. The amount of AST in the blood is directly related to the extent of the tissue damage. After severe damage, AST levels rise in 6 to 10 hours and remain high for about 4 days.

The AST test may be done at the same time as a test for alanine aminotransferase, or ALT. The ratio of AST to ALT sometimes can help to determine whether the liver or another organ has been damaged. Both ALT and AST levels can test for liver damage. Ethanol is metabolized into cytotoxic acetaldehyde by alcohol dehydrogenase in the liver and acetaldehyde is oxidized to acetate by aldehyde oxidase or xanthine oxidase giving rise to Reactive oxygen species (ROS) via Cytp 450[1,2]. Lead (Pb) is widespread toxic metals found in the environment and potential danger to human health due to its multifaceted action

with a broad range of physiological and biochemical dysfunctions [3]. It is known that Lead has detrimental effects on the central and peripheral nervous systems [4,5]. The biologically active form of vitamin B6 is required as a coenzyme in more than 103 enzymatic reactions, all six major categories of enzymes expect ligases [6,7]. Vitamin B6 is involved in a number of metabolic reactions, most of which are involved in the metabolism of amino acids and proteins, lipids, carbohydrates, nucleotide, protein synthesis and cellular proliferation[8,9]. Lead exposure induces clinicopathological changes through toxicity occurred to kidney and endocrine system . High blood lead in animals resulted in reproductive failure as it affects circulatory level of progesterone . Also, causes a decrease in reproductive fitness[10]. In the present study, the effect of lead, alcohol and vitamin E on aspartate amino transferase and alanine amino transferase on liver tissue was evaluated in *in vitro* condition.

#### MATERIALS AND METHODS

**Test Animals:** 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/ liter concentration dissolved in water. Group III animals were treated with 10% alcohol. Group IV animals were treated with 160 mg/ liter concentration of lead acetate and 10% alcohol. Group V animals served as control treated with 500mg Vitamin E/kg diet. Group VI animals were treated with lead acetate at 160 mg/ liter 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/ liter

concentration of lead acetate , 10% alcohol and 500mg Vitamin E /kg diet [11-13].

#### Estimation of aspartate amino transferase activity (AST)

**Reagents used:** Reagent 1 (enzymes) comprised of MDH  $\geq 600$  U/L, LDH  $\geq 900$  U/L, NADH 0.20 mmol/L,  $\alpha$ -ketoglutarate 12 mmol/L.

1. Reagent 1A (buffer) comprised of Tris buffer, pH 7.8, 88 mmol./L, L-Aspartate 260 mmol/L
2. Working reagent: The contents of one bottle of enzymes (reagent 1) was dissolved with the contents of one bottle of buffer (reagent 1A).

One ml of working reagent was transferred to test tube. One hundred microlitres ( $\mu$ l) of serum was added and mixed well and the absorbance was measured immediately at 340 nm. The absorbance per minute was noted. The enzyme concentration was calculated by multiplying change in the absorbance [14].

#### Estimation of alanine amino transferase activity (ALT)

**Reagents used:**

1. Reagent 1 (Buffer) comprised of Tris buffer, 110mmol, pH 7.5, L-Alanine, 550 mmol
2. Reagent 2 (Enzymes) comprised of LDH  $\geq 1200$  U/L, NADH 0.2mmol/L,  $\alpha$ -ketoglutarate 6mmol
3. Working Reagent: The contents of one bottle of Buffer (reagent 1) is dissolved with the contents of one bottle of enzymes (reagent 2)

One ml of working reagent was transferred to a test tube one hundred microlitres ( $\mu$ l) of serum was added and mixed well and the absorbance was measured at 340 nm after one minute incubation. The absorbance per minute was noted [14].

#### RESULT

**Estimation of aspartate amino transferase activity (AST):** The data on the changes in AST levels in rats treated for eight weeks are presented in (Table 1) and (Figure 1). The percent level of AST ranged from 54 to 71U/L in the control rats. In lead treated rats, the AST levels ranged from 81 to 121U/L and the increase in AST levels (Mean  $\pm$  SD, 92.50  $\pm$  19.59) were significant in lead treated rats. The increase in AST

levels (Mean  $\pm$  SD,  $100.00 \pm 16.63$ ) were marked in rats treated with alcohol compared to lead treated rats. The percent increase in AST levels was 65% in alcohol treated rats, compared to 52% in rats treated with lead. The AST levels were significantly increased in rats coexposed to alcohol and lead, and the values ranged

from 85 to 143U/L. The percent increase in lipid peroxidation was 96% in rats coexposed to alcohol and lead, compared to rats treated with either lead or alcohol. These results suggest the presence of hepatocellular damage during short-term and long term treatment with alcohol and lead.

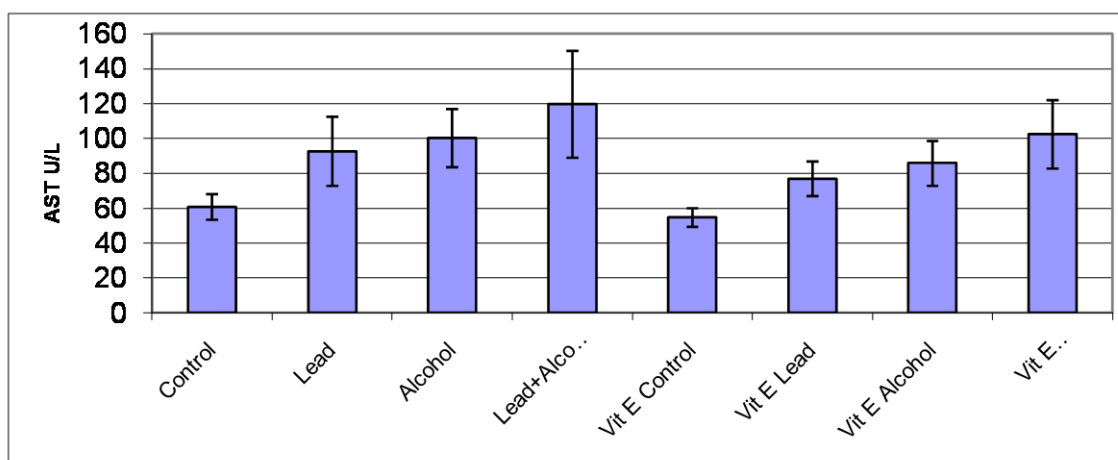
**Table 1: AST Activity in Rats Treated for Eight Weeks with Lead, Alcohol and Alcohol and Lead with and without Vitamin E Treatment**

Group	AST Mean $\pm$ SD
Control	$60.50 \pm 7.33^*$
Lead	$92.50 \pm 19.89^a$
Alcohol	$100.00 \pm 16.63^a$
Lead +Alcohol	$119.67 \pm 30.62^a$
Control +Vitamin E	$54.50 \pm 5.32 (\downarrow 9.9)^{**}$
Lead + Vitamin E	$76.75 \pm 9.95^a (\downarrow 17.0)$
Alcohol + Vitamin E	$85.75 \pm 12.82^a (\downarrow 14.0)$
Lead + Alcohol +Vitamin E	$102.25 \pm 19.70^a (\downarrow 15.0)$

<sup>a</sup> significance from control group at  $p < 0.05$

\* Values were expressed as U/L

\*\* The values in the parenthesis indicate percent change from corresponding group without vitamin E



**Figure1: AST activity in Rats Treated for Eight Weeks with Lead, Alcohol and Alcohol and Lead with and without Vitamin E Treatment**

\* Values were expressed as U/L

\* Values were expressed as percentage change over control group

**Estimation of alanine amino transferase activity (ALT) :** The data on ALT activity in rats treated with lead, rats treated with alcohol and rats coexposed to lead and alcohol for eight weeks given in (Table 2) and (Figure 2). The ALT activity (Mean  $\pm$  SD,  $33.25 \pm 4.92$ ) ranged from 29 to 38U/L in the control rats. The lead treatment was

characterized by increase in ALT activity (Mean  $\pm$  SD,  $50.50 \pm 10.47$ ). In lead treated rats, the ALT activity ranged from 35 to 58U/L. In alcohol treated rats, the increase in ALT activity was higher when compared to lead treated rats (Mean  $\pm$  SD,  $53.25 \pm 7.27$ ). The percent increase in ALT activity was 69% in alcohol treated rats compared

to 56% in lead treated groups. The ALT activity was significantly increased in rats coexposed to alcohol and lead, and the

values ranged from 59 to 81U/L. The percent increase in ALT activity was 106% in rats coexposed to alcohol and lead.

**Table 2: ALT Activity in Rats Treated for Eight Weeks with Lead, Alcohol and Alcohol and Lead with and without Vitamin E Treatment**

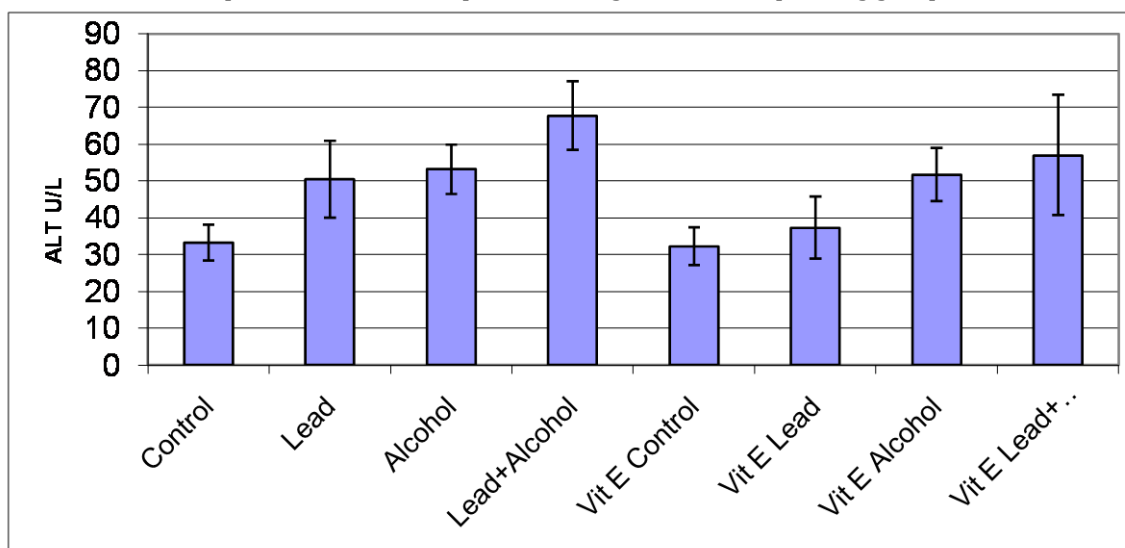
Group	ALT Mean $\pm$ SD
Control	33.25 $\pm$ 4.92
Lead	50.50 $\pm$ 10.47 <sup>a</sup>
Alcohol	53.25 $\pm$ 7.27 <sup>ab</sup>
Lead + Alcohol	67.75 $\pm$ 9.36 <sup>abc</sup>
Control + Vitamin E	32.24 $\pm$ 5.12 ( $\downarrow$ 3.0)**
Lead + Vitamin E	37.33 $\pm$ 8.51 ( $\downarrow$ 26.0)
Alcohol + Vitamin E	51.75 $\pm$ 6.70 <sup>a</sup> ( $\downarrow$ 3.0)
Lead + Alcohol + Vitamin E	57.0 $\pm$ 16.35 <sup>ab</sup> ( $\downarrow$ 16.0)

<sup>a</sup> significance from control group at  $p < 0.05$  <sup>b</sup> significance from Lead at  $p < 0.05$

<sup>c</sup> significance at  $p < 0.05$

\* Values were expressed as U/L

\*\* The values in the parenthesis indicate percent change from corresponding group without vitamin E



**Figure 2: ALT Activity in Rats Treated for Eight Weeks with Lead, Alcohol and Alcohol and Lead with and without Vitamin E Treatment**

\* Values were expressed as U/L

\* Values were expressed as percentage change over control group

## DISCUSSION

The data on the changes in AST activity at eight weeks with or without vitamin E treatment are presented in (Table 1) and (Figure 1). Vitamin E was partly effective in suppressing the AST activity in all the three groups of experimental animals. In lead treated rats, small decrease in AST activity was observed after vitamin E treatment (17%). In alcohol and vitamin E treated rats, 14% decrease in AST activity was observed. The beneficial effect of vitamin E supplementation was also seen in rats coexposed to lead and alcohol. In the

combined treatment group, 15% decrease in AST activity was observed after vitamin E supplementation.

The data on ALT activity in rats treated with lead, rats treated with alcohol and rats. After eight weeks of treatment, the magnitudes of changes in ALT activity were significantly decreased with vitamin E supplementation in all the experimental groups.

## CONCLUSION

Vitamin E was partly effective in suppressing the AST activity in all the three groups of experimental animals. In lead

treated rats, small decrease in AST activity was observed after vitamin E treatment (17%). In alcohol and vitamin E treated rats, 14% decrease in AST activity was observed. The beneficial effect of vitamin E supplementation was also seen in rats coexposed to lead and alcohol. In the combined treatment group, 15% decrease in AST activity was observed after vitamin E supplementation. After eight weeks of treatment, the magnitudes of changes in ALT activity were significantly decreased with vitamin E supplementation in all the experimental groups.

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