International Journal of Innovations & Research Analysis (IJIRA)
ISSN :2583-0295, Impact Factor: 5.449, Volume 03, No. 01(II), January- March, 2023, pp 17-19

RESTORATING ROLE OF VITAMINS IN LEAD ACETATE-INDUCED HEPATIC LESIONS IN *SWISS ALBINO* MICE

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ABSTRACT

Swiss albino mice were treated with Lead acetate (20 mg/kg wt.) in the presence (experimental) or absence (control) of both vitamins α-tocopherol (Vitamin E) 25 mg/kg and Ascorbic acid (Vitamin C) 500 mg/kg body weight. Animals were autopsied and their livers were removed at various intervals from 6 hrs to 20 days. The percentage of normal hepatocytes in both groups gradually decreased until day 2, but increased rapidly thereafter, reaching near normal values at the final autopsy interval (i.e., day 20) in the experimental group. In contrast, binucleate hepatocytes increased by day 2, then gradually decreased, returning to normal levels by day 10 in animals treated with Vitamin E + Vitamin C. The frequency of abnormal cells also increased by day 5, but decreased without reaching normal levels by the end of experiment. The counts of such cells declined significantly in mice, who received Vitamins before lead intoxication. It is concluded that Vitamin E & C can be used as preventative to inhabit lead-induced hepatic lesions in mice.

Keywords: *Lead Acetate, α-Tocopherol, Ascorbic Acid, Hepatocytes, Binucleate.*

Introduction

Heavy metals are major environmental pollutants and their toxicity is an issue of growing concern for environmental, evolutionary, nutritional and ecological reasons. (1,2). The World Health Organization's 2021 update on the effects of chemicals on Public health. Nearly half of the 2 million lives that died from exposure to known chemicals exposure in 2019 were attributed to lead exposure. Due to its non-biodegradability and continued use, its concentration in the environment built up increasing the hazard. Human exposure to lead and its compounds is primarily from industrial processes such as leaded gasoline, lead smelting and combustion, pottery, shipbuilding, lead- based coating, lead containing pipes, battery recycling, grids, defence industry, pigments, typography etc. When lead is absorbed and enters the bloodstream, it is dispersed and accumulated in various types of soft tissue in the human body. Lead accumulates in the bone, followed by the liver, kidney, neurons, and spleen (3). In March 2005, researchers at the Johns Hopkins Medical Institutions published an article in the Annals of Internal Medicine claiming that high doses of vitamin E could significantly increase mortality from all causes. Administration of vitamin C along with thiamine has been reported to significantly enhance urinary excretion of lead and reduces lead accumulation in the liver and kidneys (4). The main aim of our study was to evaluate the effects of chronic lead toxicity on the liver histology and it's prevention by vitamins.

Materials and Methods

6-8 weeks old Swiss Albino mice (22±3 g body weight), were selected from an inbred colony, maintained on standard mice feed and water *ad libitum*. These animals were divided into two groups. One group (experimental) was treated with both α-tocopherol (Vit. E) at a dose of 25 mg/kg body weight in peanut oil, intra-peritoneally and Ascorbic acid (Vit. C) 500 mg/kg body weight in double distilled water,

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orally once in a day for 7 consecutive days. The other group (control) received peanut oil and double distilled water (volume equal to vitamins) in the same manner as the drug solution. Thereafter, animals of both the groups were injected intra-peritoneally (i.p.) with lead acetate (20 mg/kg b.wt.). The dose selection for lead acetate was done on the basis of survivability experiment where mice were intoxicated with various doses of lead acetate (ie. 5, 10, 20, 30, 40 & 50 mg/kg b.wt.), and the dose of 20 mg/kg was speculated as sublethal dose. A minimum of six animals were sacrificed at intervals of 6 hrs., 12 hrs, 1, 2 ,5 , 10 and 20 days. Small pieces of liver were fixed in Bouin's fluid, 5 µm thick sections were cut using routine procedures, and stained with Harris-haematoxylene and eosin for quantitative study. Normal, bi-nucleate and abnormal hepatocytes were counted in areas measured with the help of a planimeter, and expressed as a percentage of the total number of cells counted. The values of all the types of cells were expressed as mean ± S.E., and degree of significance was determined by the Student's 't' test

Results and Discussion

- **Normal Hepatocytes:** Vitamin C and E administration before lead intoxication manifested an improved state in percentage of normal hepatocytes as compared to control group. These Vitamins rendered a significant level of protection at later intervals. The number of such cells was significantly higher than control on day 1 ($P<0.05$), 2 ($P<0.001$), 5 ($P<0.001$), 10 ($P<0.01$) and 20 ($P<0.01$). An approximately normal number of such cells was obtained on day 20 in this group (Table - 1).
- **Binucleate Hepatocytes:** Binucleate hepatic cells maintained a lower number than the control. A peak value was noted on day 2, but it remained lower than the respective control. After this, it showed a gradual decrease and attained normal level by day 10 of treatment. At 1, 2 and 5 days autopsy intervals, number was reduced significantly as compared to respective controls (Table - 1).
- **Abnormal Hepatocytes:** The number of abnormal hepatocytes increased upto day 5 alike control group, but the degree of increment was considerably lower. The percentage of these cells was significantly lesser ($P<0.05$) at 12 hrs. The protection afforded by such Vitamins was found to be significant from day 2onwards, and more or less a normal value was reappeared at day 20 (Table - 1).

Table 1: Variation in hepatic cell types (%) in mice after administration of Lead Acetate (LA) in the presence (experimental) or absence (control) of α- tocopherol and Ascorbic acid

Hepatic cell type	Treatment	Post - Treatment Autopsy Intervals						
		6hrs	12hrs	1day	2days	5days	10days	20days
Normal (90.18±0.19) #	C	89.3±0.32*****	88.0.53@	86.3±0.43@	83.8±0.31@	84.2±0.37@	86.6±0.18@	89.0±0.32@
	E	89.8±0.35	89.0±0.34	87.9±0.56	85.5±0.34@	86.3±0.32@	87.7±0.37 o	90.0±28 o
Binucleated (7.36±0.16) #	C	7.7±0.29	7.9±0.25*****	8.8±0.21@	9.5±0.20@	8.5±0.32@	7.7±0.16	7.5±0.20
	E	7.6±0.24	7.7±0.21	8.0±0.36*****	8.7±0.20 o	7.6±0.21 o	7.5±0.23	7.3±0.19
Abnormal (2.380±0.19) #	C	2.9±0.22	3.9±0.27@	4.7±0.35@	6.5±0.25@	7.2±0.22@	5.6±0.10@	3.4±0.20@
	E	2.5±0.20	3.2±0.15*	4.0±0.30	5.6±0.22 o	6.0±0.25@	4.6±0.27@	2.5±0.20o

#- Value in normal mouse (without treatment) ***** $P < 0.05$

C – Control (L A treatment) **o** $P < 0.01$

E – Experimental (α- tocopherol + Ascorbic acid + L A) @ $P < 0.001$

Lead is also known to cause liver damage by increasing oxidative stress (5). It decreases in GSH reserve and an increase in reactive oxygen species (ROS). Lead inactivates GSH by binding to sulfhydryl groups and inhibits GSH synthesis(6). In addition, lead destabilizes the cell membrane by inducing lipid peroxidation, changes the membrane's biophysical properties and causes cell damage (7). The membranous unsaturated fatty acids are the obvious targets of lipid peroxidation (8) which is caused by heavy metals, and resulting in the loss of both structural integrity and functions in the affected organelles (hepatocytic membrane). In addition to this, localized damage, the breakdown products of lipid peroxides such as aldehydes, migrate far from their production site and may cause damage at distant loci, this may be the reason for dissolution of cell membrane of two adjacent cells in lead intoxicated animals.

Vitamin E + Vitamin C pre-treated (experimental) mice showed a similar (as in lead Acetate) pattern of alteration in normal and binucleated hepatocytes number, but to a lesser extent. It indicates that such Vitamin rendered a significant level of protection at later intervals. Vitamin C is a powerful scavenger which breaks the autocatalytic process of lipid-peroxidation of membrane fatty acids, thereby preserving membrane integrity"

The percentage of abnormal cells raised up after lead acetate treatment, and it was observed as significantly higher than the normal throughout experimentation. However, the values began to decrease sufficiently from day 5 but not reinstated to normal even till the last day of post-treatment. In experimental (Alpha tocopherol + Ascorbic acid treated) mice, changes were quite similar to control, however, the percentage was significantly reduced at all the autopsy intervals. Lead acetate induces lipid peroxidation, which can damage membrane of cell organelles. Once this cell membrane is disturbed, it leads to various structural and functional alterations in the cell leading to cellular death.

A significant amount of protection has been rendered by Vitamin E and Vitamin C in the present investigation these Vitamins are presumed to occur in association with intracellular membrane. Though vitamin E is often thought of as a single compound, it's actually a group of eight fat-soluble compounds with powerful antioxidant effects (9) The chemical and biological properties of l-ascorbic acid suggest that it can act as an antioxidant *in vivo* (10). Vitamin C is a major antioxidant as it directly neutralizes free radicals. It react poorly with major cellular oxidants such as hydrogen peroxide and probably reacts mainly with the decomposition products of hydrogen peroxide (11). Vitamin C has the ability to act as a scavenger of ROS and by one-electron reduction of lipid hydroperoxyl radicals via the vitamin E redox cycle (12) Vitamin C plays role in the regeneration of vitamin E; it donates electron to tocopheryl radical (vitamin-E–O•) and reduces it to tocopherol.

It has generated interest that oxygen metabolism induces over production of free radicals due to altered pathophysiology in the system. Another hypothesis is that inadequate antioxidants in the body also impairs body defense system and ability to fight against pollutants especially heavy metal pollutants. Antioxidants can prevent cellular damage by interacting with free radicals and terminating chain reaction process (13).

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