



HISTOLOGICAL ALTERATIONS INDUCED IN MICE HEART AFTER LEAD ACETATE TREATMENT

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ABSTRACT

Lead (Pb)⁺⁺ is a persistent and common environmental contaminant that can be detected in all phases of biological system. The present study was designed to investigate the lead acetate [Pb(C₂H₃O₂)₂] induced histological changes in heart. Normal healthy looking mice showing no sign of morbidity were divided into three groups. Group I was designated as control whereas group II and group III received lead acetate having doses 10 mg/kg body weight of lead acetate, daily and 150 mg/kg body weight of lead acetate, weekly respectively. Study was performed at 40 and 80 days stages. Lead acetate given in low dose (10 mg/kg body weight) and high dose (150 mg/kg body weight) caused dose dependant changes in the anatomical cellular organization in relation to normal tissue. Treated mice at 40 and 80 day stage showed polymorphonuclear leucocyte (PMNL) infiltration which could be seen between myofibres and around necrotic sites to scavenge the damaged fibres in cardiac muscles. Clumping of nuclei to one side of vessel is also seen.

KEYWORDS: Cardiac muscles, Heart, Histopathology, Lead acetate, PMNL



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INTRODUCTION

Environmental contamination and exposure to heavy metals such as mercury, cadmium and lead is a serious growing problem throughout the world. Human exposure to heavy metals has risen dramatically in the last 50 years as a result of an exponential increase in the use of heavy metals in industrial processes and products. Among heavy metals lead is the most ubiquitous common pervasive environmental pollutant having diverse and deleterious effect on man and animal health¹. Life style factors (e.g. cigarette smoking), proximity to industrial areas, lead mines, lead based paints significantly contribute to lead pollution of the air, food, water and soil². Lead can be present in the air in vicinity of factories and before leaded gasoline was banned, it was present on highways. Vegetables are polluted by lead from the air and a considerable amount of lead contamination is found in cereals and broad – leafed vegetables³. Recently, reclamation – recycling of discarded computers has emerged as a source of heavy metals exposure among the workers involved in this process⁴. Lead becomes popular because of its dense, ductile and corrosion resistant properties⁵. Many reports are available regarding lead toxicity and deleterious effects in various species of animals. Lead is toxic agent with multiple target organs such as hematopoietic system, immune system, kidneys and nervous system⁶. Lead may be rapidly absorbed and reached in considerable amount in the blood⁷. It has been suggested that this element is strongly bound to macromolecules in the intracellular compartments because lead binding proteins have been isolated from the kidney, liver, blood and brain⁸. High doses of lead acetate caused dose dependent significant decrease in body weight⁹. Lead acetate given in high dose (640 mg/kg/day) resulted decrease in body weight during developing period of pups¹⁰. Researcher presented histological findings that characterized the degeneration and inflammatory changes in the myocardium that occurred in conjugation with chronic lead poisoning¹¹. Administration of lead, most commonly as lead acetate salt, to experimental

animals has been shown to induce myocarditis, degenerative structural changes affecting musculature of heart¹². The present study was conducted to investigate histological alterations in heart after lead acetate treatment.

MATERIALS AND METHODS

The present investigation was carried out on heart of adult sexually mature Swiss albino mice weighing 20 – 30g. They were maintained in polypropylene cages under hygienic conditions with proper temperature and light. Mice were fed upon Hindustan lever pellets diet and water *ad libitum*. All experimental procedure was conducted after approval of Institutional Animal Ethics Committee (IAEC/Bio/6-2011) of H. P. University, Shimla.

CHEMICALS

All reagents used were of highest grade. Lead acetate used for this study was obtained from Sigma Chemicals, St. Louis, MO, USA.

GROUPING OF ANIMALS AND DOSE ADMINISTRATION

Mice were divided into three groups: Group I served as control, group II received oral administration of lead acetate (10 mg/kg body weight) daily and group III administered lead acetate (150 mg/kg body weight), weekly. Lead acetate was given for 40 days and mice were sacrificed at 1, 40 and 80 days period.

HISTOLOGICAL STUDIES

Heart was excised, fixed in Bouin's fixative and dehydrated in different grades of alcohol. Finally, sections were embedded in paraffin wax and were subjected to hematoxylin – eosin staining.

RESULTS

HISTOPATHOLOGICAL CHANGES

The control group depicted long branched muscle fibers with long, cylindrical nuclei (Fig 1). Mice treated with lead acetate for 40 days

showed significant toxic changes in the heart. At 40 days stage, mice heart given low dose of lead acetate, demonstrated a little distortion around blood capillaries and clumping of nuclei towards one area. Fragmentation of fibres was also observed. (Fig 2). Mice given high dose of lead acetate revealed polymorphonuclear leukocyte infiltration to scavenge the necrotic tissue along with some hypertrophied muscle fibres and enucleated muscle fibres. There is merging of fibres with some lysed fibres that further lead to myonecrosis. Blood capillaries with distorted outline along with pycnotic nuclei

can be seen (Fig 3). At 80 days stage, after withdrawal of drug at 40 days, the histological examination of heart depicted recovery towards normal with little effects. In some sections, however the recovery was not so evident and still the degenerated muscle fibres along with pycnotic nuclei were noticed. Clumped nuclei and nuclear streaks were also seen around the blood vessel (Fig 4). Some sections showed changes in shapes of nuclei and they became spindle and needle shaped. Capillary with highly compressed lumen were also observed in section (Fig 5).

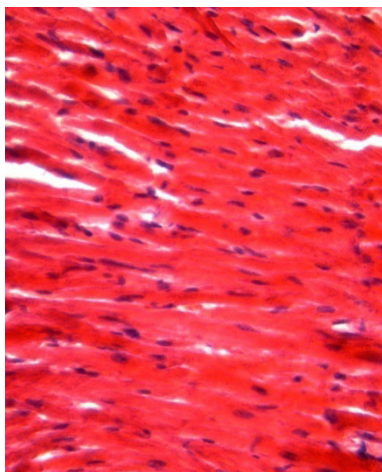


Figure 1
T.S. of normal mice heart showing long fibres with centrally placed nuclei x 400

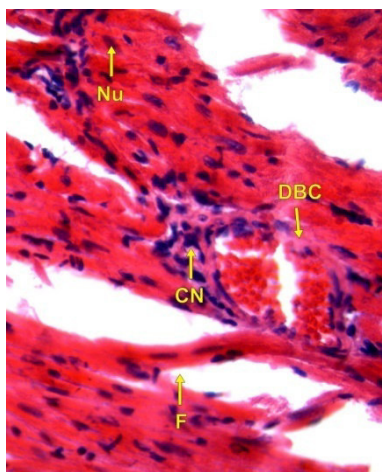


Figure 2
L.S. of lead acetate (10 mg/kg body weight) treated mice heart depicting distortion around blood capillaries (DBC) and fibres showing fragmentation (F) and clumping of nuclei (CN) is seen near the blood vessel x 400

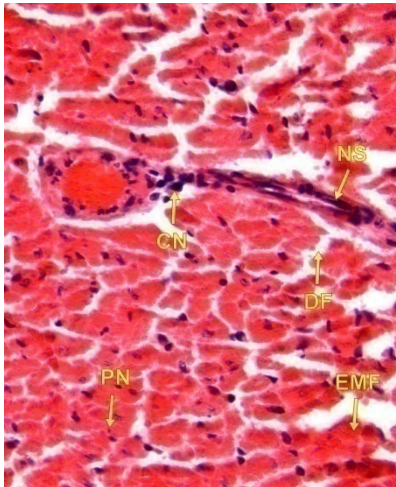


Figure 3

T.S. of weekly lead acetate treated (150 mg/kg body weight) mice heart at 40 days, showing maximum myofibre degeneration (DF). Nuclei get clumped (CN) on one side of blood vessel and form nuclear streaks (NS) in interfibrillar spaces. Enucleated muscle fibres (EMF) and some pycnotic nuclei (PN) are also noticed x 400

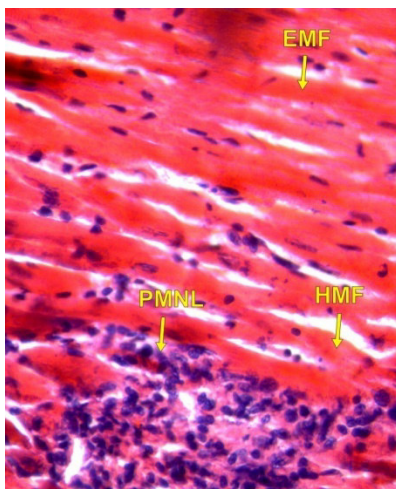


Figure 4

L.S. of lead acetate treated mice at 80 days after dose withdrawal at 40 days, demonstrating polymorphonuclear infiltration (PMNL). Hypertrophied muscle fibres (HMF) and enucleated muscle fibres (EMF) are also noticed x 400

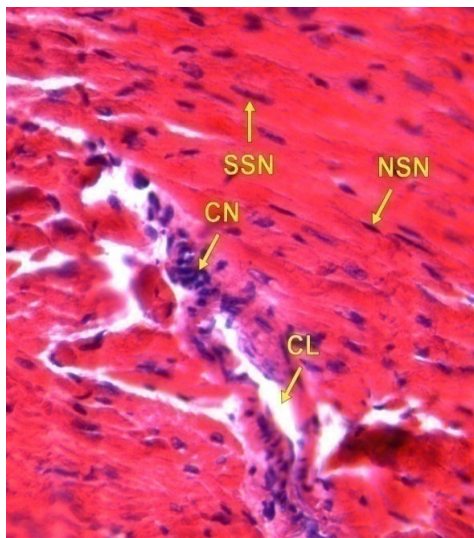


Figure 5

L.S. of treated mice heart at 80 days after dose withdrawal at 40 days, depicting blood capillary with highly compressed lumen (CL). Spindle shaped nuclei (SSN) and needle shaped nuclei (NSN) can also be noticed x 400.

EFFECT ON BODY WEIGHT

Daily and weekly doses of lead acetate caused a significant decrease in the average body weight. In the present study, 10 – 15% of body weight were found to be decreased at 40 days, whereas after the withdrawal of lead acetate at 40 days, the body weights come towards normal level although a slight decrease of 2 – 4% was noticed as compared to control.

DISCUSSION

Lead toxicity is the most studied health problem in the recent years. Lead is toxic in most of its chemical forms, whether it is inhaled or ingested via water or feed. The extent to which orally administered lead absorption is small, however, due to its slow rate of elimination, harmful level of lead can accumulate in tissues after prolonged exposure in low quantities^{13, 14}. Animal and tissue weights were measured to determine whether the results obtained were confounded by any nutritional factors. Mice given lead acetate exposure for a 1 day period maintained almost constant body mass. Our results were supported by the findings of researchers, who revealed that the exposure of lead acetate for 15 or 45 days produced a significant decrease in mice body mass, but after 90 days of exposure mass reversed to

initial values suggesting a remarkable lead poisoning resistance in mice¹⁵. Similar results were observed by other scientist also¹⁶. Lead exposed animals showed increased lipid peroxidation and decrease in antioxidant defense mechanisms^{17, 18}. Further results showed that rats treated with lead for up to 12 weeks exhibit significant hypertension (HTN) which is accompanied by oxidative stress and elevated plasma lipid peroxidation (LPO)¹⁹. Heart is a fibromuscular organ. Fibrous part of heart is formed of extracellular matrix (ECM) whereas its muscular part is comprised of elongated muscle cells. Toxic heavy metals increase the acidity of blood. Further, they set up conditions that lead to inflammation in arteries and tissues, causing more calcium to be drawn to the areas as a buffer, contributing to hardening of the artery walls with progressive blockage of the arteries and osteoporosis. Lead toxicity affected the normal histological structure of heart and caused disturbances in its normal function. Heart lesions as a result of lead treatment were in the form of hemorrhage, myocardial necrosis, mononuclear cell infiltration, fibrosis were seen and myocardial muscle revealed signs of myocardial necrosis, loss of striation and karyopycnosis²⁰. In the present study, all control and treated mice were observed carefully for the appearance of any

toxic signs. At 40 day stage, mice administered with low dose of lead acetate revealed fragmentation of fibres. Outlines of vessels were found distorted with clumping of nuclei at one area. Mice given high dose of lead acetate demonstrated myofibre degeneration. Sections showed nuclear streaks in interfibrillar spaces. At the 80 days stage, after lead acetate withdrawal at 40 days, sections depicted polymorphonuclear leucocyte infiltration. Other

sections exhibited variously shaped nuclei like spindle and needle shaped.

CONCLUSION

Thus, this study showed that lead acetate given in low dose (10 mg/kg body weight) and high dose (150 mg/kg body weight) caused a dose dependant changes in the anatomical cellular organization in relation to normal tissue.

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