International Journal of Research in Engineering, Science and Management
Volume-1, Issue-11, November-2018
www.ijresm.com | ISSN (Online): 2581-5792

Structural Changes in Mice Gastrocnemius Muscle after Lead-Acetate Treatment

Sushma Sharma[1], Anita Thakur[2]
[1]*Professor, Department of Biosciences, Himachal Pradesh University, Shimla, India*
[2]*Ph.D. Scholar, Department of Biosciences, Himachal Pradesh University, Shimla, India*

***Abstract*: The present study was designed to investigate the lead acetate [$Pb(C_2H_3O_2)_2$] induced histological changes in gastrocnemius muscle. Lead (II) acetate is a white, crystalline, water soluble solid used as a mordant in textile printing and dyeing. 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 1, 40 and 80 days stages. Histopathological examination of haematoxylin stained heart sections revealed that most of the significant pathological lesions were found in lead acetate treated mice. At 40 days stage, lead acetate treated mice gastrocnemius muscle fibres in affected areas showed vigorous invasion of poly morpho nuclear leucocytes (PMNL) which encircle the atrophied fibres. Extreme variations in the nuclei were also seen varying from pycnotic to hypertrophy. Degenerating connective tissue was also noticed. However at 80 days stage an overall better fibre histo architecture was noticed after lead acetate withdrawal as compared to treated ones.**

***Keywords*: lead acetate, histopathology, gastrocnemius muscle.**

1. Introduction

Life style factors (e.g. cigarette smoking), proximity to industrial areas, lead mines, lead based paints and leaded gasoline significantly contribute to lead pollution of the air, food, water and soil (Kasuba et al., 2004). Lead toxicity is closely related to its accumulation in various tissues and its interference with bio elements that hamper several physiological processes (Pande et al., 2001; Barrahal et al., 2007). In humans the lead is directly absorbed, distributed and excreted. Lead dust, fumes or vapors are more easily absorbed from the respiratory tract. Once lead enters the body it interferes with normal cell functions and physiological processes. It affects the RBCs and cause damage to organ including liver, kidney, heart and male gonads as well as also affects the immune system (Patel, 2000). After absorption into the blood, 99% of lead is bound to erythrocytes and the remaining 1 % stay in the plasma to be carried to the other tissues. Lead finds its way to hard tissues like bone and teeth, where it accumulates, only to result in sustained release and maintenance of an unacceptable blood lead level, many years after the exposure period (Rio et al., 2001; Popovic et al., 2004; Smith, 2008). Skeletal muscle contributes approximately 40% of total body mass. Its solid constituent is nearly all proteins (about 20% of the whole). The remaining 80% or so is water in which various minerals P, Ca, Mg etc. are dissolved. Skeletal growth is an integral component and primary stimulator of somatic growth. The terminally differentiated cells of skeletal muscles are very large, multinucleated muscle fibers. Muscle fibers form when mono nucleated myoblasts cease replicating and fuse with each other to form multinucleated cells that express muscle specific proteins such as myosin and other members of the contractile apparatus (Stockdale, 1997; Miller et al., 1999). In humans, the gastrocnemius muscle is very powerful superficial pinnate muscle that is in the back part of the lower leg. It runs from its two heads just above the knee to the heel, and is involved in standing, walking, running and jumping. Gastrocnemius's most important role was planter flexing in large contractions and in rapid development of tension.

2. 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.

A. Chemicals

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

B. 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.

C. 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.

D. Histological results

Haematoxylin – eosin stained gastrocnemius muscle of normal mice showed purple colored nuclei (N) in subsarcolemal position. Fiber heterogeneity was clearly seen (Fig: 1). Mice treated with lead acetate showed significant changes in histo architecture of gastrocnemius muscle. At 40 days stage mice gastrocnemius muscle treated with low dose of lead acetate depicted polymorphonuclar leucocyte (PMNL) infiltration around myofibres. Migration of nuclei towards center was observed. Interfascicular spaces were noticed. Various shapes of muscle fibers such as triangular and rectangular were visualized clearly (Fig: 2&3). Mice given high dose of lead acetate revealed degenerated connective tissue around blood vessel. Purple colored nuclei were visible at subsarcolemmal position. Inter fascicular spaces were large however no inter fibrillar spaces were seen. Some sections showed enucleated muscle fibers with extrusion of nuclei to inter fibrillar spaces (Fig: 4&5). At 80 days stage, after withdrawal of lead acetate at 40 days, an overall better histoarchitecture was observed as compared to control. Interfibrillar spaces were small with few degenerated muscle fibres. Some atrophied and hypertrophied muscle fibers could be noticed. Little merging of fibers was also observed (Fig. 6, 7).

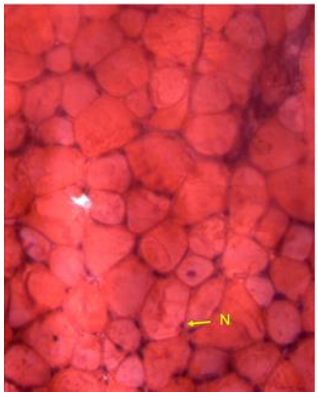

Fig. 1. T.S. of normal mice gastrocnemius muscle depicting compactly arranged muscle fibres with normal well stained nuclei (N) X (400)

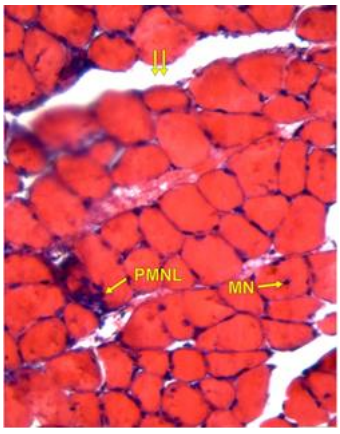

Fig. 2. T.S. of lead acetate (10 mg/kg body weight, daily), treated gastrocnemius muscle after 40 days showing polymorphonuclear leucocytes (PMNL) infilteration around myofibres. In certain fibres, the nuclei shifted their position deep inside the fibres towards the centre (MN) X (400).

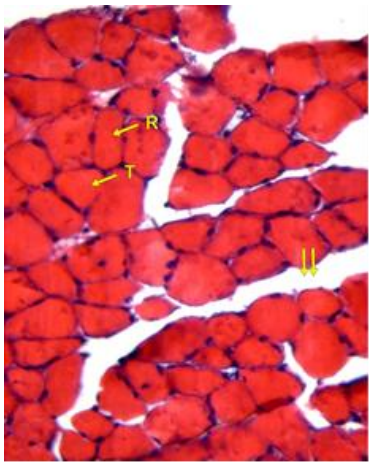

Fig. 3. T.S. of lead acetate (10 mg/kg body weight daily), treated mice gastrocnemius muscle after 40 days exhibiting large interfascicular spaces (↑↑). Various shapes of muscle fibres such as triangular (T) and rectangular (R) were also noticed X (400).

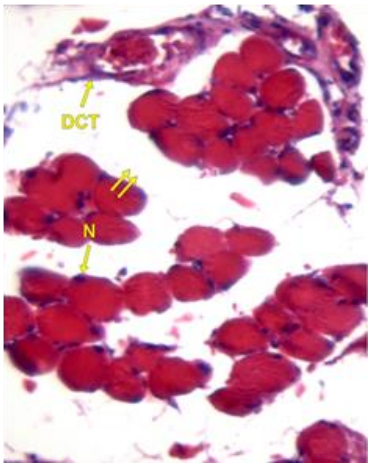

Fig. 4. T.S. of lead acetate (150 mg/kg body weight) administered mice muscle after 40 days depicting the blood vessels with degenerating connective tissue (DCT). Large interfascicular spaces can be seen (↑↑) X (400).

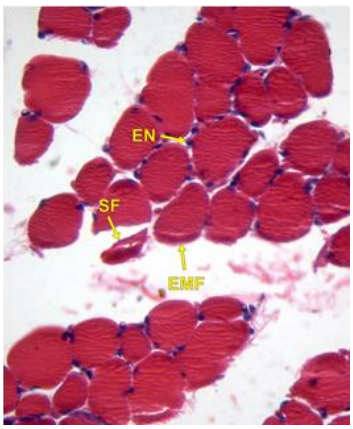

Fig. 5. T.S. of lead acetate (150 mg/kg body weight, daily), treated muscle after 40 days demonstrating the enucleated muscle fibres (EMF) with extrusion of nuclei (EN) to interfibrillar spaces. Splitting of fibres (SF) in clear parts is also visible X 400.

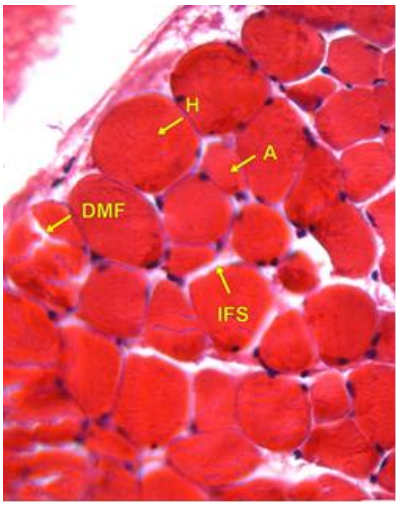

Fig. 6. T.S. of gastrocnemius muscle at 80 days after withdrawal of lead acetate at 40 days exhibiting atrophied (A) as well as hypertrophied (H) muscle fibres, small interfibrillar spaces (IFS) with a few degenerating muscle fibres (DMF).

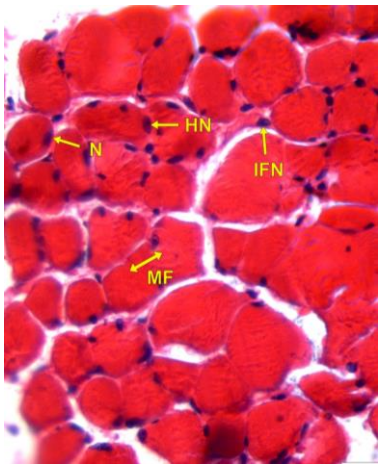

Fig. 7. T.S. of gastrocnemius muscle at 80 days stage after withdrawal of lead acetate at 40 days depicting merging of fibres (MF). Some of nuclei are sub-sarcolemmal in position (N) while others are present in interfibrillar spaces (IFN). Hypertrophied nuclei (HN) are also seen X (400).

3. Discussion

Lead is considered as one of the most hazardous and cumulative environmental pollutant that affects all biological systems. Lead is absorbed via respiratory tract, gastrointestinal tract and occasionally through the skin. Lead absorption via respiratory tract is highly efficient, resulting in an average uptake of 40% of inhaled lead (Fischbein, 1998). Lead may be rapidly absorbed and reached in considerable amount in blood (Haque et al., 2006). It has been suggested that this element is strongly bound to macromolecules in the intracellular compartment because binding proteins have been isolated from the kidney, liver, blood and brain (Han et al., 1996; Moussa et al., 2001). Lead affects bone development and mineralization and has antagonistic activity in calcium metabolism (Kim et al., 2000; Gavazzo et al., 2008). A study has been made on the effects of lead on developing muscle using a cell culture system. When L6 clonal cell line was treated with lead acetate in culture during the first or second day after plating fusion of cells to form skeletal muscle straps, was inhibited. This inhibition was not due to a general toxic effect of lead on the cells since cell division continued during the first 10 days of culture after lead addition. When cells were examined ultra-structurally, the only apparent effect of lead was a change in mitochondrial size and configuration. Five times condensed mitochondria were seen in lead treated cells and mitochondrial size was also significantly increased. Lead appears to affect a very early event in cell fusion since gap junction formation and some synthesis of myofibres occur even in the presence of lead (Harary and Berliner, 1980).

Lead toxicity affected the normal histological structure of gastrocnemius muscle and caused disturbances in normal functions performed by them. In present investigation, the histological alterations in the gastrocnemius muscle of mice caused by lead exposure were observed. To demonstrate histological changes in control and treated stages, haematoxylin and eosin stain was used. Sections of gastrocnemius muscle were evaluated under light microscopy. All the control and treated mice were observed carefully for appearance of any toxic signs. Dose – dependent changes were observed in muscle. At 40 day stage, mice administered with low dose of lead acetate showed migration of nuclei from peripheral to central position. Hypertrophy of myofibres with large interfascicular spaces was also seen. Mice given high dose of lead acetate demonstrated degenerating connective tissue. Enucleated muscle fibers with extrusion of nuclei to interfibrillar spaces was also noticed. At 80 days stage, after lead acetate withdrawal at 40 days, depicted less adverse effects in gastrocnemius muscle. Interfascicular spaces were much reduced as they were in 80 days stage, however, small interfibrillar spaces were seen at places. Little hypertrophy of fibers was also noticed. To evaluate the effect of long-term exposure of heavy metals on skeletal muscle, chronic subcutaneous injections for 7 days of two lead treatments (low dose, 0.1 mg/kg of body weight and high dose, 1 mg/kg of body weight) of lead acetate were investigated. The high dose of chronic lead treatment induced ultra-structural changes, including reduced number of synaptic vesicles, disruption of mitochondria and increased number of smooth endoplasmic reticulum and myelin – like structures in the intramuscular axons and neuromuscular junctions. Chronic lead treatment caused extensive disruption of the sarcoplasmic mitochondria and increased the number of myelin – like figures in the muscle. These results suggested that exposure to lead at a low concentration can compromise in situ skeletal muscle isometric concentration (Dhaheri et al., 1996).

4. Conclusion

Thus, this study revealed that histological examination of gastrocnemius muscle showed dose dependent signs of lead acetate induced degenerative changes in relation to normal tissue.

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