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Consequence of lead on the histology of the cerebellum of the brain of adult Albino rat

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ABSTRACT

Background: Lead is one of the common environmental contaminant. Lead poisoning can affect the various cognitive and behavioral function of the brain and cerebellum is one of the main part of brain concerning to the cognitive function. That's why this study is intended to find the Consequence of lead on the histology of the cerebellum of the brain of adult Albino rat.
Methods: Twelve adult albino rats of Charles Foster strain (6 experimental and 6 control groups) were used as the experimental animal in the present study. After 21 days exposure of lead, albino rats were sacrificed and tissue perfusion was done. Sectioning of tissues and H & Ex staining was done and viewed under microscope.
Results: swollen and degenerated Purkinje cells which were decreased in number, Some pyknotic cells showed pericellular clearing. Numerous hyperchromatic and pyknotic granular cells were also seen with edematous molecular cell layers and showed spongiosis of the grey matter with focal proliferation of capillaries and necrosis.
Conclusion: Grey matter showed spongiosis and focal proliferation of the capillaries along with necrosis.
Keywords: Lead, cerebellum, Albino rat, brain, gray matter.

INTRODUCTION

Environmental contamination of metallic elements can affect the several parts of body of human and animals [1]. Among these metallic elements, lead (Pb) is one of the most harmful to living organisms [2]. Lead poisoning is not a new incident [3]. It is think over that the adverse effects of such a wide spread use of lead among the Romans may have contributed to the eventual fall of the empire [4]. The metal is primarily found in leaded gasoline, paints etc [5]. Due to its non-biodegradable nature and continuous use, its concentration accumulates in the environment with increasing hazards. Lead, which is a soft, grey blue heavy metal, is a common cause of poisoning in domestic animals throughout the world [6].
Lead is a poisonous metal, which exist in both organic (Tetraethyl lead) and inorganic (lead acetate, lead chloride) forms in the environment [7]. Lead exposure is unavoidable as it exposed through many routes, i.e. air, water, soil, food, and consumer product [8]. Even at low levels of lead exposure there are functional and structural impairments in humans and experimental

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animals [9]. The biological effects of lead acetate have been centered principally due to its properties as a highly toxic sustainable poison in humans and animals [10]
Lead is highly toxic and can interrupt the body's neurological, biological and cognitive function. Children are particularly susceptible as per the WHO and high levels of lead exposure can cause brain, liver, nerve, and stomach damage, as well as permanent intellectual and developmental disabilities [11]. Lead toxicity causes irreversible health effect. It hampered a number of body functions by primarily affecting the central nervous, hematopoietic, hepatic and renal system, causing serious disorders [12].As special attention has been paid to health hazards of environmental pollution, the aim of the present work is to find consequence of lead on the histology of the cerebellum of the brain of adult Albino rat.

MATERIAL AND METHODS

Procurement and selection of animals

Twelve adult albino rats of Charles Foster strain (6 experimental and 6 control groups) were used as the experimental animal in the present study. They were kept on balanced diet acquired from the Central Animal House. Ethical approval was taken from institutional ethical committee.

Experimental Protocal

A total of 12 adult albino rats weighing 280gm approximately were used in the present study. Out of 12 rats, 6 rats were treated with 4% lead acetate, while the other 6 served as controlled animals and were given distilled water. Freshly prepared sterile 4% solution of lead acetate in distilled water was used for oral administration, as drinking water. This concentration was ascertained after a careful trial in order to find maximum survival days, which were 15 to 21 days.

Sacrifice and perfusion of animals

Rats were anaesthetized with ether. An intravenous infusion set was used consisting of a bottle, a drip chamber and 6-7 feet of rubber tubing supplied with squeeze type flow regulator the tubing was connected to canula. The entire assembly was checked for airlock. 10% formalin solution in normal saline was used as the perfusion fluid. The animal was laid on its back on a dissecting tray with secured extremities. The thorax of the anaesthetized animals was opened by an incision starting at the base of xiphoid process and extending along the median line of the sternum to the jugular notch avoiding the internal mammary artery that runs close to the sternum. The opening of the thorax was widened by cutting symmetrically along intercostal spaces on each side, starting from xiphoid process the flaps of the thorax wall formed in this was clamped and rolled upward.
An eighteen gauge needle introduced into the ascending aorta through the left ventricle. The right atrium was widely opened and the perfusion by formal saline started at a pressure of about 5 feet of water pressure. Perfusion was stopped when head and tail stiffness became pronounced. Proper perfusion was ascertained by the fasciculation on extremities and tail and oozing of clear fluid drops from the snout of the animal.
The animal was decapitated and skin as well as all soft tissue surrounding the cranium was removed. The cranial vault was removed with the help of pointed scissors starting from the sides of the foramen and extending anterolaterally. After the superior and lateral surfaces of the brain were uncovered the brainstem was lifted from the base of cranium, simultaneously severing the attached nerves and vessels.

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Following removal, the intact brain was examined for any macroscopic changes. Meningeal coverings were removed; subsequently the cerebellum was cut and transferred to specimen tubes containing formalin (10%) for the next 48 hours in order to allow for the fixation of the perfusion fixed brain.

Preparation of Tissue Block

Cerebellum was thoroughly washed with distilled water and dehydrated by placing an increasing strength of alcohol (50%, 70%, 90% and absolute alcohol) for duration of one and a half-hour each. Then dealcoholization was done by clearing agent, i.e. xylene. Two changes of xylene, each for two hours was found adequate for making the tissue clear and translucent. Then it was placed in molten wax for embedding or impregnation of tissue with wax for two hours. For casting into blocks "Leuckhart's L- pieces were used. Rapid cooling was done for solidification of the wax blocks by immersion into a tray of cold water.

Cutting of Paraffin Blocks and Mounting on Glass Slide

The paraffin blocks were trimmed and fixed to block holder and with the help of rotatory microtome 10mm thick sections were cut. Adequate length of the ribbons of the section was lifted and transformed to tissue floating hot water bath. After flattening, the sections were lifted on slides which had before been smeared with Meyer's egg albumin. The slides with mounted sections were dried in an incubator at 40-45°C.

Methods of Staining

Sections from both the control and lead acetate treated rats were stained by the following staining techniques:-

Haematoxylin and Eosin Stain Reagents

- Alcohol in different strengths (absolute alcohol in 3 jars 90% alcohol and 70% alcohol in two jars each). Separate jars were used for hydration and dehydration.
- Xylene (in 3 coplin jars for removing wax two for clearing of stained sections).
- Harris's Haernatoxylin: 20g potassium alum was dissolved in 200ml warm distilled water in a (one liter) conical flask. 1.0g haematoxylin was dissolved in 10ml absolute ethanol and were added to the alum solution. The mixture was rapidly brought to the boiling point and 0.5g mercuric oxide was added. The stain was rapidly cooled by plugging the flask into cold water and was filtered.
- Eosin solution (1.0%)
- D.P.X (mountant)
- Cover slips

Procedure

- Removal of wax with xylol.
- Hydration of sections, using descending grades of alcohol.
- Staining with haematoxylin and eosin.
- Dehydration with ascending grades of alcohol (70%, 90% and 100%).
- Clearing was done by two changes of xylene, each for two hours.
- Mounting in D.P.X. and a cover slip.
- The sections were deparafinized with xylene and transferred to absolute alcohol. They were hydrated with descending grades (90%, 70% and 50%) of alcohol to water. The sections

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stained with haematoxylin 3-5 minutes were placed in running tap water until they turned blue. Counter staining was done with 1% aqueous eosin for 30 seconds. The sections were dehydrated with ascending grades of alcohol cleared in xylene (two changes) and mounted in D.P.X.

OBSERVATION AND RESULTS

Histological changes of the cerebellum

In the control group all the three layers of the cerebellum were clearly visible on Haematoxylin and Eosin staining i.e. Outer molecular, inner granular cell layer and single layer of Purkinje cells in between the two layers at 10 X (Fig. no. -1a & 1b) and 40 X (Fig. no.-1c).

In the experimental group, stained section of the cerebellum with Haematoxylin and Eosin showed the swollen and degenerated Purkinje cells which were decreased in number. Some pyknotic cells showed pericellular clearing. Numerous hyperchromatic and pyknotic granular cells were also seen with edematous molecular cell layers 10X (Fig. no.-2a).Similarly, in another low power showed the vacuolar degeneration of the molecular cell layer with separation from the granular layer. The Purkinje cells were shrunken with an irregular Pyknotic nuclei at 10X (Fig. no.-2b). In (Fig. no.-2c) 10X of the experimental group, showed spongiosis of the grey matter with focal proliferation of capillaries and necrosis. Reduced numbers of Purkinje cells with dark rounded nuclei were also observed. Marked vacuolar degeneration and edema were seen in the granular cell layer.

At high power magnification, marked vacuolar degeneration of all the cell layers with degenerated Purkinje cells were observed and rounded configuration of nuclei with perinuclear clearing at 40X (Fig. no.-3a) was also noticed. Section exhibited vacuolar and cellular degeneration of all the cell layers, with dark and rounded nuclei along the shrunken purkinje cells. The granular cell layer showed proliferation of capillaries with pyknotic nuclei 40X (Fig. no.-3b).

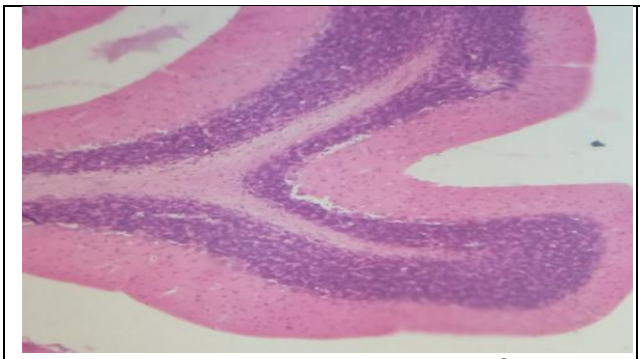

Fig. no. 1a- Photomicrogaph of the cerebellar cortex of adult Albino rat (Control group) showed all the three layers i.e. molecular, Purkinje layer and granular layer.H&Ex10X.

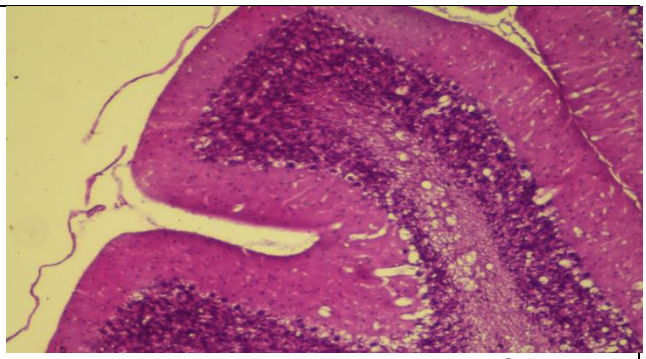

Fig. no. 1b- Photomicrograph of the cerebellar cortex of albino rat (control) showed all the three layer with unremarkable histomorphology. H&Ex10X.

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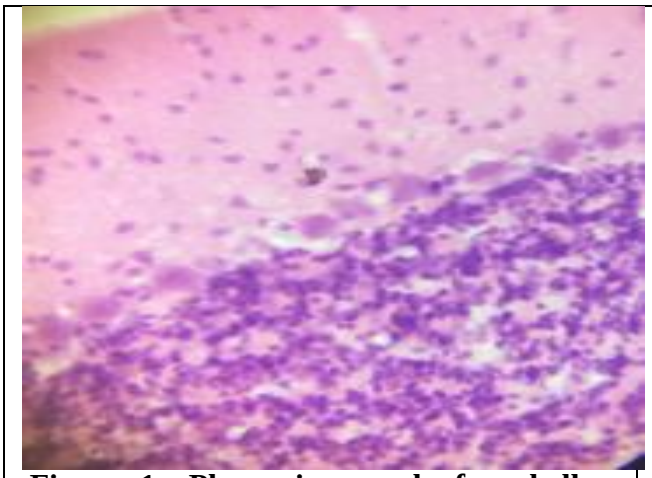

Fig. no. 1c- Photomicrograph of cerebellar cortex of adult albino rat (control) showed three layers with unremarkable histology. H&Ex 40X.

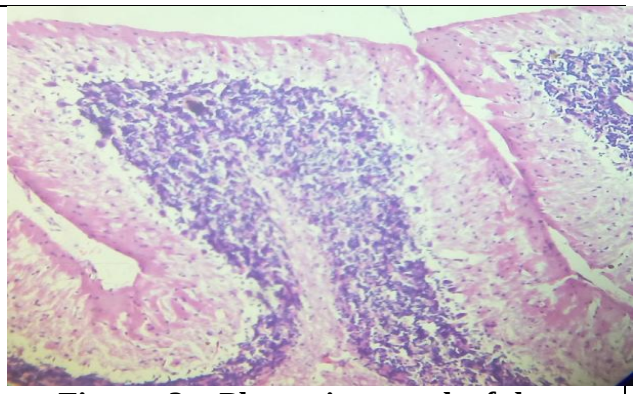

Fig. no. 2a- Photomicrograph of the cerebellar cortex of adult Albino rat (Experimental group) showed swollen and degenerated Purkinje cells which are decreased in number. Some pyknotic cells show pericellular clearing. Numerous hyperchromatic and pyknotic granular cells also seen with oedematous molecular cell layers. H&Ex10X.

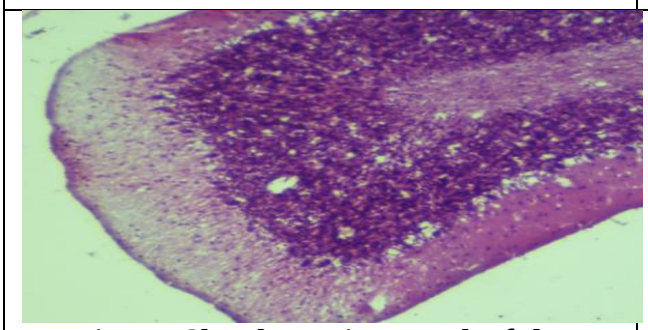

Fig. no. 2b- Photomicrograph of the cerebellar cortex of albino rat (EXPERIMENTAL) showed vacuolar degeneration of the molecular cell layer with separation of the granular layer from the molecular layer. The Purkinje cells are shrunken with an irregular pyknotic nucleus. H&Ex10X.

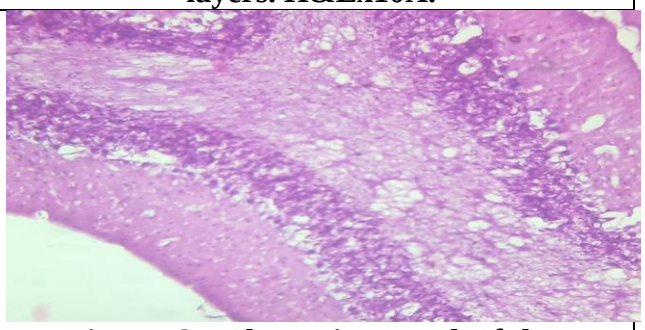

Fig. no. 2c- Photomicrograph of the cerebellar cortex of the albino rat (experimental) showed marked vacuoler degeneration of all the cell layers with degenerated Purkinje cells which shows the rounded configuration of nuclei with perinuclear clearing. H&E 10X

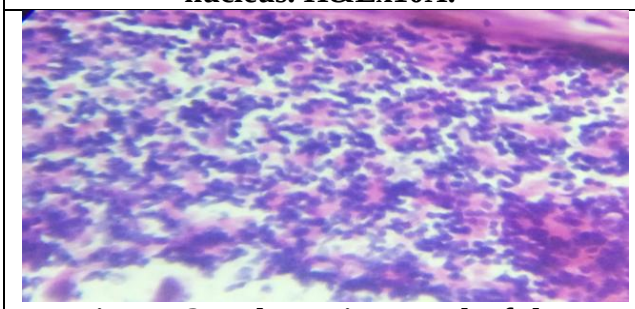

Fig. no. 3a- Photomicrograph of the cerebellar cortex albino rat (experimental) showedspongiosis of the gray matter with

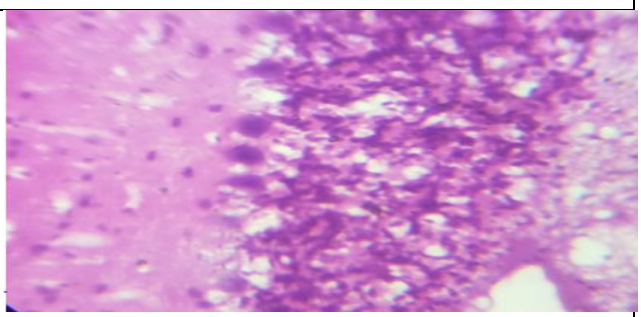

Fig. no. 3b- Photomicrograph of the cerebellar cortex of adult albino rat (

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the focal proliferation of capillaries and necrosis. Reduced number of Purkinje cells with dark rounded nuclei seen. Marked vacuolar degeneration and edema seen in the granular cell layer. H&Ex40X.	**experimental) showed vacuolar and cellular degeneration of all the cell layers with shrunken Purkinje cells with dark and rounded nucleus. The granular cell layer shows proliferation of capillaries with Pyknosis of nucleus. H&Ex 40X**

DISCUSSION AND CONCLUSION

The present study is aimed at qualitative analysis of the histological features of the cerebellum of the brain. Twelve adult albino rats, weighing 280 gm approximately and about 3 months of age, was to be obtained from the central animal house. They had kept on balanced diet obtained from the Central Animal House. Rats were divided into group A and group B containing 6 rats each. Group B (Experimental group) rats were given lead acetate solution in distilled water orally while Group A (Control group) were given distilled water orally.

By the end of third week rats were sacrificed and brain was obtained by dorsal approach. Grossly the brain looks edematous and numerous petechial hemorrahges were observed on their surface. The region of the Cerebellum sectioned with paraffin, and stained with Haematoxylin and Eosin, to observe the different histological layers of the cerebellum.

Lampert et al., reported that the weight of the Cerebellum was markedly decreased in suckling rats after chronic lead exposure [13]. At the end of treatment schedule of this experiment, there was marked reduction of the body weight of experimental group was observed in comparison to control animals. Similar observations were also reported by in their studies [14-16].

Press MF who performed the histological study of many parts of the brain e.g. cerebral cortex, corpus striatum, choroid plexus and the cerebellum after lead exposure, revealed cerebellum to be most severely damaged. In addition to this study, hemorrhages were noticed along with damage to molecular and Purkinje cell layers and edema in the granule cell layer which were correlated very well with the findings of the present study [17].

Various studies reported the degeneration of the cells of the cerebral cortex. And reduced numbers of the Purkinje and granule cells of the cerebellum after lead exposure on guinea pig. Similar findings were observed in the present study like cerebral vascular proliferation with swollen neurons and gliosis. Purkinje cells in the cerebellum were also reduced in numbers and shrunken with irregular pyknotic nuclei [18-19].

In the Cerebellum lead was mainly accumulated in white matter rather than cortical grey matter, probably due to vascular damage in the cerebral cortex, which results edema fluid transported in cerebellum or directly passages were formed in the Cerebellar white matter, these findings were partially agreed with our observations [20].

The cerebellum after lead exposure showed a marked alteration in its layers, mainly the molecular layers width decreases, granule cell layer decreases in density and arborization of dendrites were observed, total weight of the cerebellum were also decreases**.** Due to vacuolar degeneration of the molecular layer, the granular layer separated from the molecular layer, which causes decrease width of the molecular layer, which was reported in the present study [21].

The present study showed Shrinkage of the Cerebellar Purkinje cells with empty spaces around them in lead exposed rats. The degeneration of Purkinje cells was in agreement with other studies done by [21-23].

The following histological changes of the cerebellum of the brain were found-

a) Purkinje cells were shrunken, degenerated and reduced in numbers.

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b) Granular cells were hyperchromatic and pyknotic.
c) Granular cell layer were separated from molecular layer and vacuolar degeneration were noticed in the molecular layer.
d) Grey matter showed spongiosis and focal proliferation of the capillaries along with necrosis.
e) Degenerated Purkinje cells showed rounded configuration of the nuclei with perinuclear clearing.

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