

A COMPARATIVE STUDY OF THE HISTOLOGICAL CHANGES IN CEREBRAL CORTEX, HIPPOCAMPUS, CEREBELLUM, PONS & MEDULLA OF THE ALBINO RAT DUE TO LEAD TOXICITY

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ABSTRACT

Introduction: Lead, a heavy metal is well known for its toxic effects on the central nervous system. Clinically, overall effects of lead on different organ system are called plumbism. Diverse writing can be seen on the subject, but rarely there has been a comparison in any of these writings on different parts within the brain of the changes happening as the result of lead exposure. This study was taken up to draw a comparison and correlation of damaging effects on different parts of brain at microscopic level as a result of lead toxicity so that the affected elements in the tissue can be further connected to the histopathological and clinical outcomes of the lead toxicity.

Materials and Methods: To conduct the study albino rats of Charles Foster strain were administered orally with 4% lead acetate in drinking water. The behavioral and clinical changes during the period of lead administration were closely observed that extended from irritability, agitation and aggressive behavior in the beginning to drastic fall in activity, indifference towards varieties of stimulus and severe motor deficit. At the end of an average of 17 days the rats were sacrificed for both gross and microscopic examination of brain for changes in the cerebral cortex, hippocampus, cerebellum, pons & medulla. The elements of the tissue observable as per the selected staining were the neurons, fibers, glia & the vessels.

Results: The changes showed up with similarities between different parts as the shrinkage of neurons, damaged fibers, stunting of cell processes and increased glial cell population, whereas there were dissimilarities with regards to the extent of shrinkage of neuron and distribution of perineuronal spaces, vacuoles & the glial cells.

Discussion and Conclusion: The comparative picture of the changes as a result of lead exposure showed widespread damage to nearly all the elements of the nervous tissue with reactive changes e.g. gliosis, and variations in the extend of changes in the selected brain parts. As a result these changes observed can be of used to correlate in the overall outcome of plumbism in relation to the functions of different parts of the brain.

KEY WORDS: Lead neurotoxicity, albino rats, Charles Foster, lead acetate, cerebral cortex, hippocampus, cerebellum, pons, medulla, pyramidal cells, Purkinje cells, CA1.

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INTRODUCTION

"If we were to judge the interest excited by any medical subject by the number of writings to

which it has given birth, we could not but regard the poisoning by lead as the most important to be known of all those that have been treated up to the present time." – Orfila [1]

Lead is a heavy metal well known from the history of mankind to the present day for its diverse uses, misuses and the toxicity. Even after the enactment of laws and regulations against the damaging use still there are sporadic cases where the excess amount of lead is being reported in the consumables and environment.

Once ingested orally in the food, from the environment or in mother's milk to infants the lead is slowly absorbed in the gastrointestinal tract [2] Inhalation or transdermal routes can also serve as the other forms of intake. After consumption, the toxicity at cellular level happens and it is believed that it is due to the effect on mitochondrial phosphorylation and related enzymes. Additionally, it can act as a competitive inhibitor of the Ca transport at the level of Ca pump and channels and that's how the lead induces its toxicity leading to the disturbance in the cellular homeostasis.

The clinical manifestation of the lead toxicity popularly known as plumbism depends on the level of exposure, affinity of various tissues and the route of intake. As a consequence the lead accumulation happens in different tissues at different concentrations, but the sequestration in the nervous tissue although lesser in amount from circulating lead produces more crippling effects leading to physical disabilities and even disorders of the higher functions of the central nervous system.

The studies so far have shown variability in effect on the different parts of brain on lead exposure. The chronic lead effects on exposure to the parietal cortex of Wistar rats [3] has shown microglial cells assuming characteristic spindle or rod shape, widening of endoplasmic reticulum, appearance of inclusion bodies and hypertrophy of microglial cells as well as vascular pericytes with other tissue elements intact. In a separate study [4] it was found that there were no changes in the blood capillaries with acute lead exposure and was inferred the primary toxicity effects happen at neuronal level reflected with encephalopathy. Vacuole formation at the tips of gyri with accompanying gliosis and capillary activation has also been observed by some workers [5]. McConnell et al [6] found reduced densities of granule and Purkinje cells, with reduction in dendritic length

and abnormality in branching pattern.

Zook et al [7] with experimental lead paint poisoning induced in primates hippocampus showed degenerative and proliferative changes of small vessels, ring hemorrhages, edema, perivascular hyaline droplets, rosette-like deposits of proteinaceous exudate, focal loss of myelin, astrogliosis and necrosis of neurons. Brinck et al [8] observed that the pyramidal neurons of the CA1 region and the granule cells of the fascia dentata were well preserved in the center, whereas neuronal structures in outer parts were either vacuolated or hyperchromatic and shrunken. Most of the CA4 neurons were lytic. Morphometric analysis of the pyramidal cells of CA1 yielded approximately 55% well preserved pyramidal neurons.

Patrick and Anderson [9] studied the lead effect on the cerebellum and the result revealed the increase in spine density and altered patterns of dendritic branching. Complex dendritic branching was evident, with a progressive shift in peak branching peripherally. Lead-exposed Purkinje cells showed early sprouting with subsequent pruning. The lead induced effect was evident on dendritic height, width and distance from the surface of the cerebellum.

Considering the disparities in observations, to confirm the findings, to include the various parts of brain and to look for similarities and differences in the lead induced effect the study was taken up to investigate the changes in selected parts of the brain due to lead toxicity.

MATERIALS AND METHODS

The organic lead exposure was studied on the albino rats of Charles Foster strain. A total of 12 adult albino rats weighing 120gm (+/- 10gm) were used in the present study. Six rats of either sex were treated with 4% lead acetate solution in drinking water; this concentration was ascertained after a careful trial in order to find maximum survival days, which were 16 to 18. The other six rats served as control and were given normal drinking water orally. After 17 days they were sacrificed and formalin (10%) fixed by perfusion method. The brain was exposed and removed from cranium. Following removal the intact brain was examined for macroscopic changes. Meningeal coverings were removed and

about 3mm thick coronal slices of cerebrum, cerebellum and brain stem (medulla oblongata) were sectioned and transferred to specimen tubes containing 10% formalin for the next 48 hours in order to allow additional fixation of the perfused brain. The slices of different parts were further processed for paraffin embedding and tissue blocks were prepared. The section of 6µ thickness were cut and mounted for special tissue staining for light microscopy.

RESULTS

Lead toxicity presented various aspects of behavioral and clinical effects. Effects appeared from third day onwards ranging from irritability, agitation and aggressive behavior in the beginning to drastic fall in activity, indifference towards varieties of stimulus, motor deficit to the extent of paralysis in few rats. The exposed brain showed gross edema with petechial hemorrhages on the surface.

The cerebral cortex of experimental group showed reduced number of stained neurons in experimental group. The neuron somata appeared shrunken with stunting of basal dendrites reduced branching and decreased area of arborization compared to the control. The glial cell population showed higher number but the cell variety cannot be distinguished. Further, there was enormous increase in vacuoles with respect to size and number reflecting the affected neurons and the neuropil.

Nissl preparation shows in the experimental group an overall reduction in thickness of *hippocampus* affecting all and markedly the pyramidal layer where the neurons are less distinct and poorly identified. The Kluver Barrera stained sections shows highly vacuolated region in the zones of the deep pyramidal layer. Overall there is gross neuronal degeneration and reduced neuronal population over the entire pyramidal layer.

The lead treated cerebellum showed features very similar to that of cerebral cortex and hippocampus with loss of neurons in different layers of cerebellar cortex. Sections showed swollen as well as degenerating Purkinje cells with widening of perineuronal space. There was substantial reduction in overall population of Purkinje cells, which constitutes major efferent

source of cerebellum. The granule cell layer also had reduced cell population with presence of empty space in this layer.

As compared to the control in experimental group the section of the *pons* showed loss of neurons, breaking of pontocerebellar fiber bundles and separation of individual fibers leading to loss of distinct orientation. The descending pathways appeared swollen degenerated with empty space in their mapped position. The overall impressions of changes were the neuronal damage, fiber degeneration, edema and disorganization of pontine cytoarchitecture.

The experimental sections of medulla oblongata revealed changes almost similar to the pons but relatively less marked. In control the transversely cut fiber bundles show normal axis cylinders, the same were degenerated in the fiber bundles of the sections from lead exposed medulla.

Fig. 1: Low power micrograph of golgi stained cortical neurons from control showing multipolar neuron (pyramidal) with profuse arborisation.

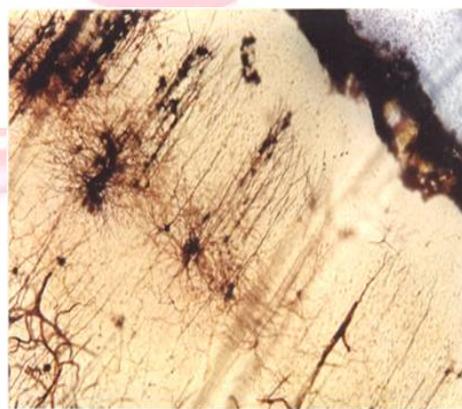


Fig. 2: Low power micrograph of Golgi stained cortical neurons from experimental showing multipolar neuron with shrunken soma and stunted dendritic arborisation. Glial proliferation is also marked compared to control.

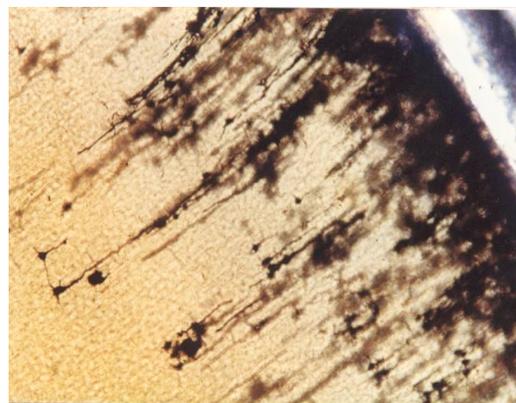


Fig. 3: High power micrograph of Nissl stained section of cerebral cortex from control; showing features of a large multipolar neurons having vesicular nucleus, prominent nucleolus and well developed Nissl substance, standing out from that of glial cells.

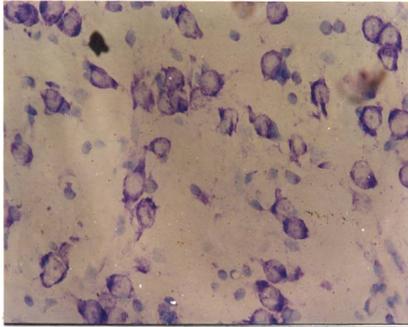


Fig. 7: Low power micrograph of Nissl stained section of hippocampus from control; showing well defined neuronal cell layers. Neurons have features of characteristic large multipolar neurons.

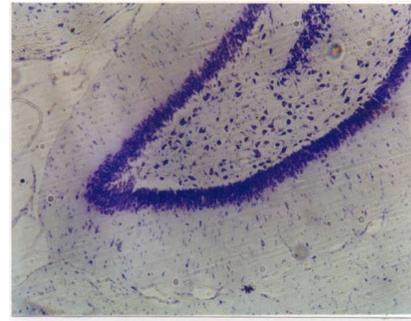


Fig. 4: High power micrograph from Nissl stained sections of cerebral cortex from experimental; showing shrunken neurons (characteristic features of neuron subdued) compared to control.

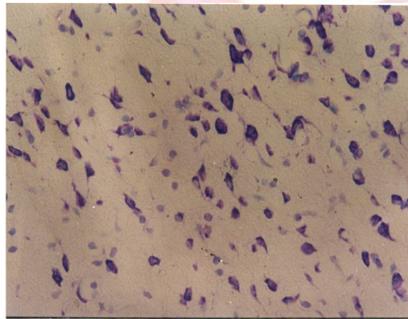


Fig. 8: High power micrograph of Kluver-Barrera stained section of hippocampus from experimental; showing highly vacuolated region in the zones of the deep pyramidal layer.

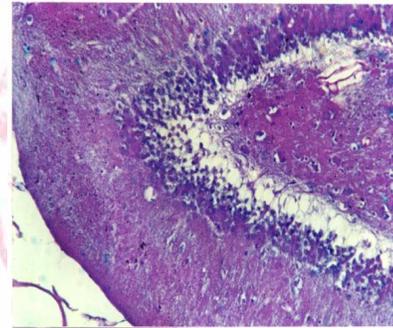


Fig. 5: Low power micrograph from Gleys Silver stained section of cerebral cortex; except for minor dehydration artefact, showing normal histology and occasional light stained spots suggestive of capillary.



Fig. 9: High power micrograph of Nissl stained section of cerebellum from control; showing characteristic three lamina. The neuron in the densely packed granular layer cannot be resolved separately.

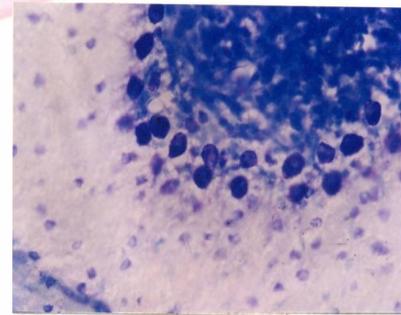


Fig. 6: Low power micrograph from Gleys Silver stained section of cerebral cortex; showing uniformly stained spots throughout the cortex suggestive of vacuolation and vascular proliferation.



Fig. 10: High power micrograph of Nissl stained section of cerebellum from experimental; Purkinje neurons showing loss of Nissl substance and granular layer showing loss of neuron and neurons can be identified separately. Some neurons showing perineuronal space.

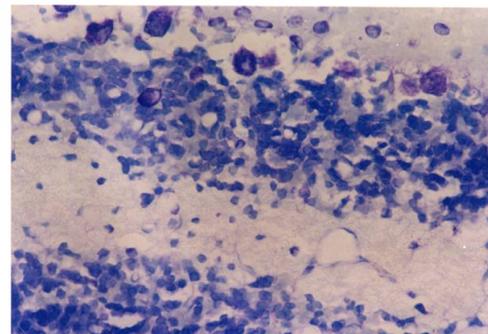


Fig. 11: Low power micrograph of Gleys Silver stained section of pontine region from control well stained bundles of pontocerebellar fibers are mingled with lightly stained bundles of ascending and descending tracts. Darkly stained collection of neurons (pontine nuclei can also be seen).



Fig. 12: Low power micrograph of Gleys Silver stained section of pontine region from experimental; pons showing complete loss and breaking of darkly stained fibres leading to disorganization of the pontine cytoarchitecture.

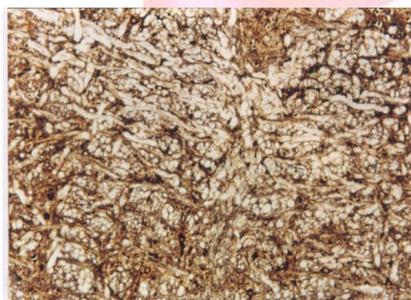


Fig. 13: High power micrograph of Gleys Silver stained section of medulla from control; showing well stained fibres with distinct orientation prolonged continuity. Ascending and descending tracts are well packed.

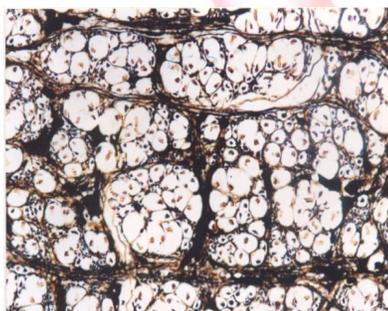
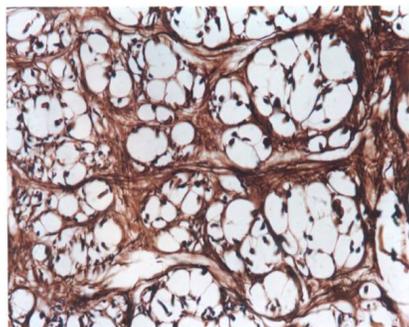


Fig. 14: High power micrograph of Gleys Silver stained section of medulla from experimental; As compared with control changes are not very much marked however fibres are relatively lightly stained and tract regions appear to be swollen.



DISCUSSION

Lead is a neurotoxin and this fact is well corroborated by this study in which adult albino rats of Charles Foster strain were exposed to lead in the drinking water. Signs of toxicity were evident as early as third day with irritability progressing to agitation and aggressiveness with culmination in twitching, tremors to the extent of quadriplegia in two of the lead treated rats.

Shrunken neuron was almost a universal occurrence but the degree of effect was variable in different parts of brain. The affected pyramidal neurons in the cortex were irregularly distributed with same density as in control without loss of neurons or change in neuronal density [6]. In the hippocampus the entire layer of neurons was almost uniformly shrunken but qualitatively did not show any loss [8]. The cerebellum showed reduced population from granular cell layer, decreased size of Purkinje cells but not to the extent as seen in the size of pyramidal cells of cerebral cortex and there was accompanying loss of Nissl substance from Purkinje cells. In the pons almost complete disappearance of neurons was seen and the accompanying extensive damage to the fibers resulted in a complete disorganization of cytoarchitecture. The effects in medulla were similar to the pons.

The effect on fibers differed in various regions. The dendritic processes of the cortical neurons showed stunting with reduced arborization and since the branching was substantially reduced due to the damage therefore the branching pattern [6] was not observable. The partially viewed white matter showed paler staining due to reduced density of fibers as a result of damage. No obvious damage to the fibers associated with the pyramidal neurons in the hippocampus was observed in the lead treated sections. In the pons the organization of fibers is disarrayed & loss of continuity due to the damage is obvious.

The experimental cortical sections has increased number of glial cells in agreement with Stowe et al [5] but without the presence of spindle shaped cells and hypertrophy of pericytes [3]. The cerebellum did not showed any such change in the population of glial cell. The vacuolation

was reflected in experimental group extensively as pale spots dispersed almost uniformly throughout the thickness of cortical layer, contrary to the observation were the distribution of vacuoles was on tip of gyri [5], besides the presence of perineuronal space reflecting the extent of damage to the neuron in the cerebral cortex. Lead treated hippocampal section showed highly vacuolated area as a layer limited to the zone of deep pyramidal layer and not the outer part of hippocampus [8]. In the cerebellum the perineuronal space was observed related to some neurons only.

CONCLUSION

On the basis of the observations there is no doubt that lead is a neurotoxin. It affected all the selected parts of the brain in the current study i.e. cerebral cortex, hippocampus, cerebellum, pons & medulla. The changes were observed in neurons, glia, fibers and vascular structures with appearance of vacuoles and perineuronal spaces. Although the size of neurons diminished in cerebral cortex, hippocampus and cerebellum with appearance of perineuronal space but it differed in various parts. The increased glial population with appearance of vacuoles was especially evident in cortical areas of the cerebrum and the damage with disarray of fibers as a consequence of damage in pons & medulla. Therefore, it is expected that the outcome of the effects clinically will be reflected as a dysfunction as not just a result of any individual damaged part but it will show up as a shared effect of different parts of the brain.

Conflicts of Interests: None

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