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A Histological and Histochemical Study of the Cerebral Cortex Following Acute Lead Poisoning in Wistar Rats

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ABSTRACT

The effect of acute lead poisoning on the cerebral cortex of wistar rat was investigated. Twenty adult wistar rats weighing 200-220g were randomly divided into four groups designated A, B, C and D with five rats in each group. Group A served as control and received food and water *ad libitum* throughout the experimental period. Groups B, C and D were given food and water contaminated with 100ppm, 1000ppm and 5000ppm of lead acetate respectively *ad libitum* for 21days. At the end of the experiment, the animals were sacrificed using chloroform anesthesia. The whole brains were fixed in formol saline; cerebral cortex was then dissected and processed for routine H and E staining and Nissl substance using cresyl fast violet method. Results showed dose dependent weight loss ($P<0.05$). Histologically, the cerebral cortex of group B rats showed hypertrophied cells, group C showed vacuolations around cells with few cells at various stages of cell death while group D showed hypertrophied cells with many cells at various stages of cell death. There was also dose dependent chromatolysis in the treatment groups stained for Nissl substance when compared with the control. These results revealed that the toxic effect of lead exposure is dose dependent.

Keywords: Lead Acetate, Cerebral Cortex, Nissl Substance, Body Weight.

Introduction

Lead poisoning is a medical condition caused by increased level of the heavy metal lead in the body. No safe threshold for lead exposure has been discovered. Thus, there is no known amount of lead harm (Rossi, 2008). Lead poisoning occurs when a person swallows, absorbs or inhales lead in any form (Beer *et al.*, 2004).

Lead is one of the most important toxic metals due to its increasing level in the environment as the result of industrial practices (Jarub, 2003). Lead poisoning

may be acute (intense exposure of short duration) or chronic (repeat low level exposure over prolonged period) but the latter is much more common (Trevor *et al.*, 2007).

Causes of environmental contamination include industrial use of lead such as is found in facilities that process lead acid batteries or produce lead wire or pipes (Manay *et al.,* 2008). In adults occupational exposure is the main cause of lead poisoning (Needleman, 2004). People can be exposed when working in facilities that produce a variety of lead containing products; these include radiation shields, ammunition, certain surgical equipment, fetal monitor, plumbing circuit boards, jet engines and ceramic glazes (Patrick, 2006). Parents who are exposed to lead in the work place can bring lead dust home on their skin or clothes and expose it to their children (Dart *et al.*, 2004). Some lead compounds are colourful and are used widely in paint (Heuretig, 2006) and lead paint is a major route of exposure in children (Gilbert *et al.,* 2006). Lead acetate is used as a reagent to make other lead compounds and as a fixative for some dyes, in low concentration. It is the principal active ingredient in progressive types of hair colouring (Paradyot, 2002).

The surface of the cerebrum including its gray matter forms the cerebral cortex. Thus, the cerebral cortex is a layer covering the surface of the cerebral hemispheres. It is only 2-3mm thick and constitutes about 40% of the mass of the brain. Neural integration is carried out in the gray matter of the cerebrum which is found in three places, the cerebral cortex, basal nuclei and limbic system (Kenneth and Lesile, 2004).

This study was therefore designed to investigate the effect of acute lead poisoning on the histology and Nissl substance of the cerebral cortex.

Materials and Methods

Chemicals: Lead II acetate was procured from a reputable chemical store in Onitsha, Nigeria. The chemical was dissolved in water to produce the following concentrations, 100ppm, 1000ppm and 5000ppm.

Experimental Animals: Twenty male albino wistar rats weighing 200 – 2220g were procured from the animal house of the Department of Hematology, University of Nigeria Teaching Hospital Enugu, Nigeria. The animals were allowed to acclimatize for two weeks in the animal house of the Department of Anatomy, Anambra State University, Uli Campus. They were fed with standard feed and water *ad libitum* and maintained under standard laboratory conditions

(temp. 26 ± 2^0C and 12hrs natural dark cycle). Random sample was used to divide the rats into four groups of five animals each.

Experimental Design: Group A rats served as control group and were given food and water *ad libitum* throughout the experimental period. Groups B, C and D were given food and drinking water contaminated with 100ppm, 1000ppm and 5000ppm of lead acetate respectively *ad libitum* for 21 days.

On the 22nd day, the animals were sacrificed using chloroform anesthesia. The brains were quickly extracted by opening the skill to have access. The whole brains were preserved using formal saline, when properly fixed, the cerebral cortex was excised and processed for routine H & E stain and Nissl bodies using cresyl fast violet.

Statistical Analysis: The mean body weight of the rats of each group was taken before and after the experimental period. The data was subjected to statistical analysis using student t- test. The experimental values were expressed as mean ± standard error of mean (SEM). Values of probability $P<0.05$ was taken to be statistically significant.

Results

a. **Body Weight:** There was a dose dependent decrease in body weight in the treatment groups as shown in table 1 below:

Group	No. of Rats	Doses (ppm)	Mean Body Weight Before Experiment	Mean Body Weight After Experiment
A	5	Control	210.0 ± 7.10	230 ± 11.64
B	5	100	208.8 ± 3.96	202 ± 4.15
C	5	1000	213.4 ± 3.91	204 ± 1.00
D	5	5000	202 ± 11.11	190 ± 3.08

b. **Histological Studies**: The cerebral cortex of control group showed normal histology with numerous cells in the marginal, cortical, intermediate, subventricular and ventricular layers. The cerebral cortex of group B rats given drinking water contaminated with 100ppm of lead acetate for 21days showed fewer cells in the cortical zone and cells in the intermediate and suventricular layers appeared larger in size when compared with the control. Cerebral cortex of group C rats given drinking water contaminated with 1000ppm of lead acetate showed few small vacuolations around the cells and many cells showing karyoherexis in the subventricular zone.

Cerebral cortex of group D rats given drinking water contaminated with 5000pm of lead acetate for 21 days showed larger vacuolations around cells and cells at various stages of cell death. The cells also appeared hypertrophied when compared with the control.

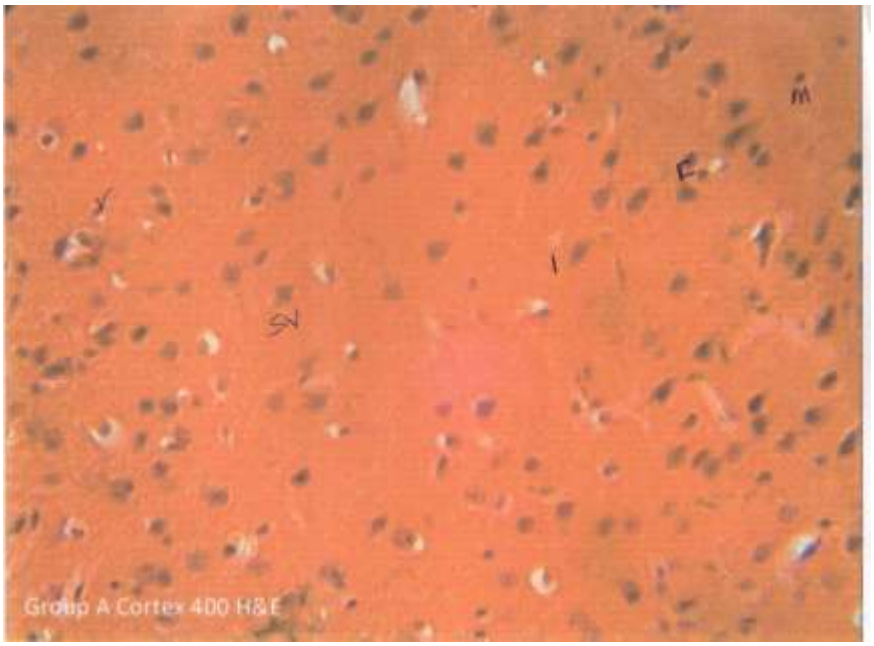

Plate 1: Photomicrograph of Control Group Showing Normal Histology of the Cerebral Cortex.

Key:

M - Marginal
C - Cortical
I - Intermediate
Sv - Subventricular
V - Ventricular layers

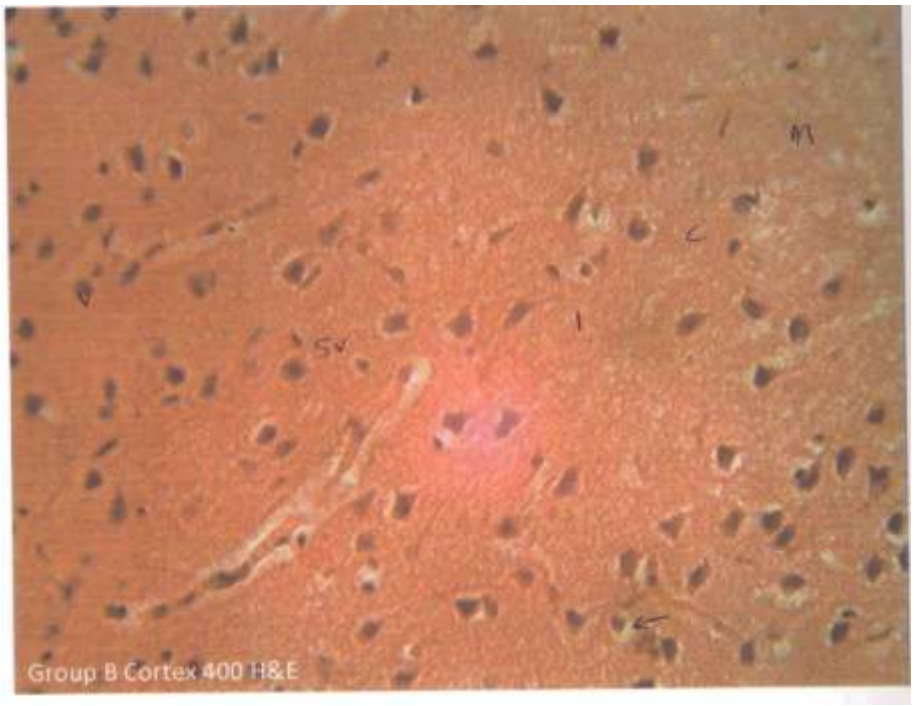

Plate 2: Photomicrograph of Cerebral Cortex OG Group B Given Water Contaminated with 100ppm Lead Acetate Showing Hypertrophied Cells in the Intermediate and Subventricular Layers.

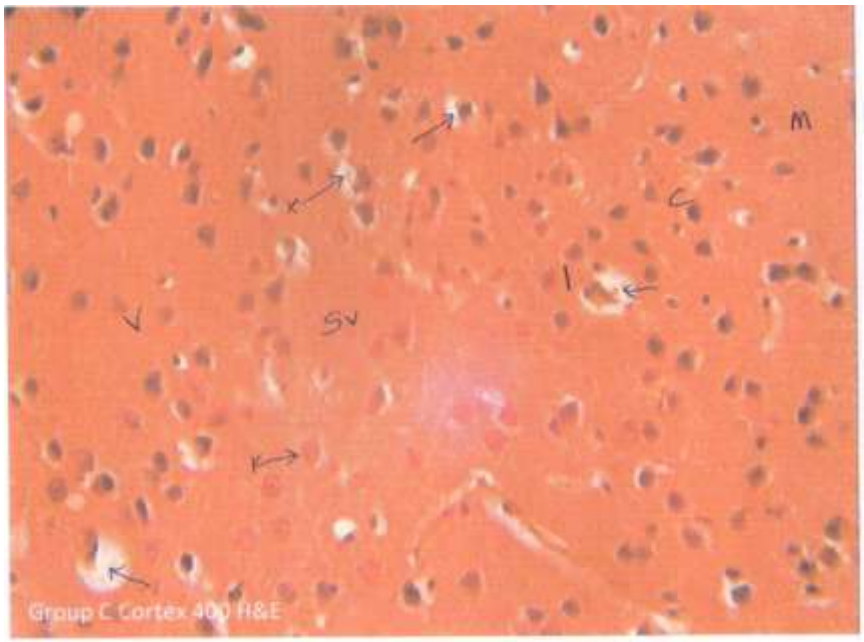

Plate 3: Photomicrograph of Group C Cerebral Cortex Given Water Contaminated with 1000ppm Lead Acetate Showing Vacuolations Around Cells and Many Cells Showing Karyoherexis in the Subventricular Zone.

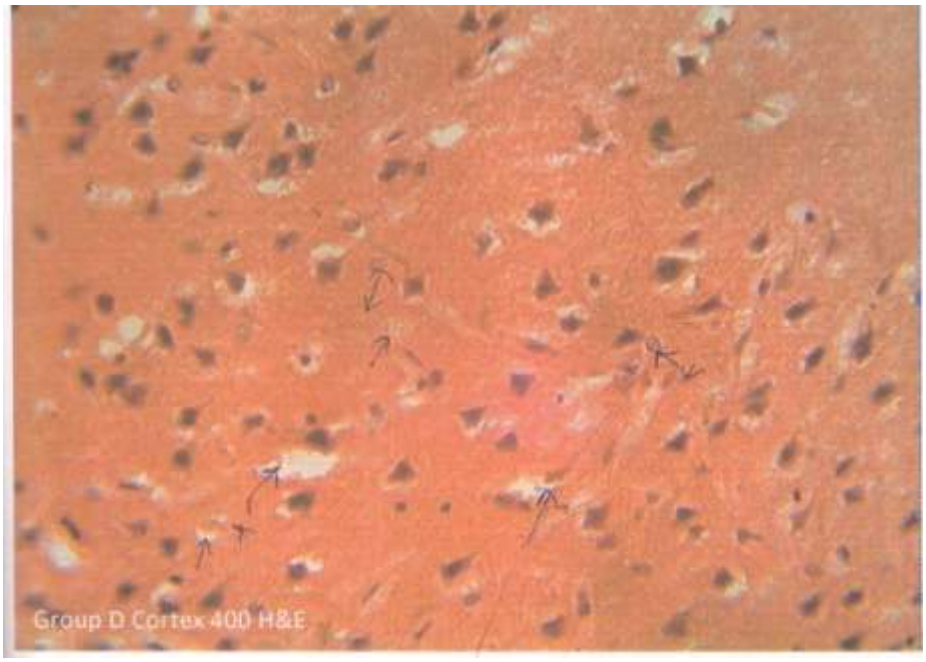

Plate 4: Photomicrograph of Cerebral Cortex of Group D Given Water Contaminated with 5000ppm Lead Acetate Showing Cells at Various Stages of Cell Death.

c. **Histochemical Studies (Nissl Substance):** Nissl substance was deeply demonstrated in the cerebra of control and group B rats. Nissl substance was demonstrated in cerebral cortex of group C rats with few neuronal cells exhibiting chromatolysis. More neuronal cells exhibited chromatolysis in the cerebral cortex of group D rats.

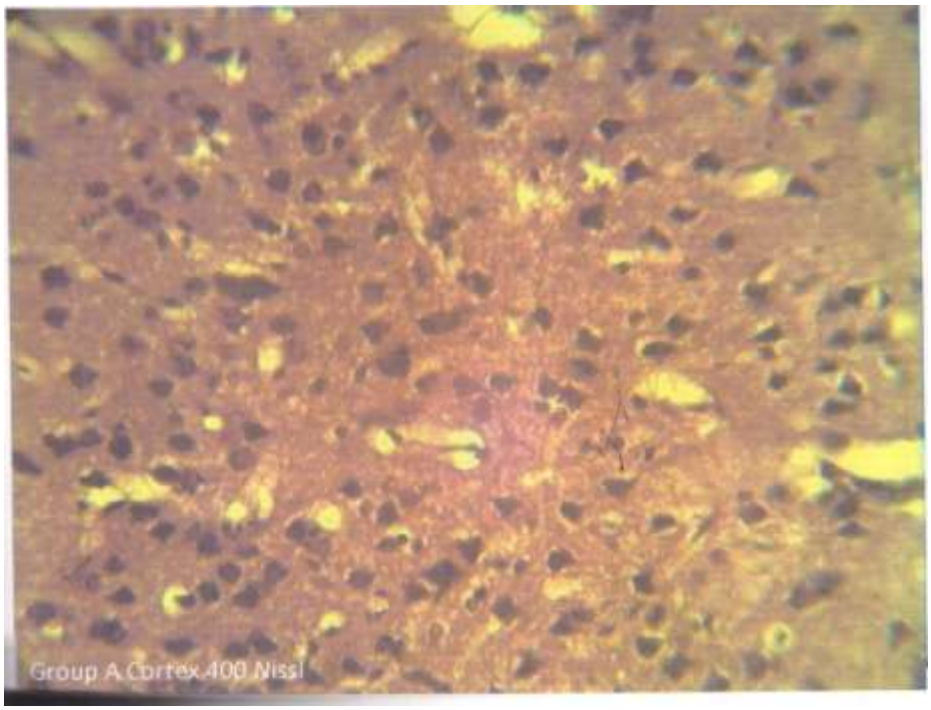

Plate 5: Photomicrograph of Control Group Showing Numerous Neuronal Cells Demonstrating Nissl's Substance

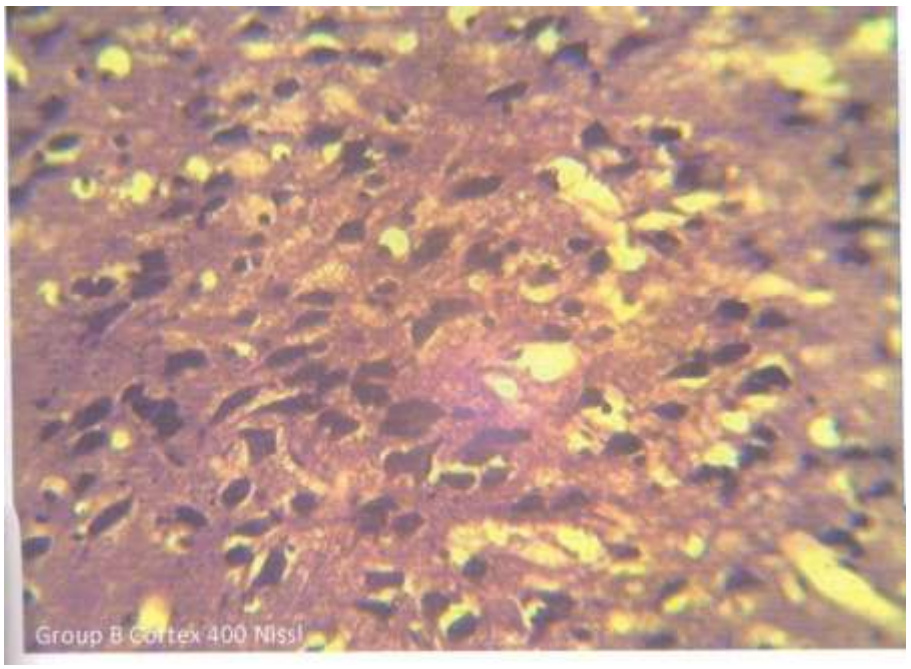

Plate 6: Photomicrograph of Group B Showing Numerous Neuronal Cells Demonstrating Nissl's Substance

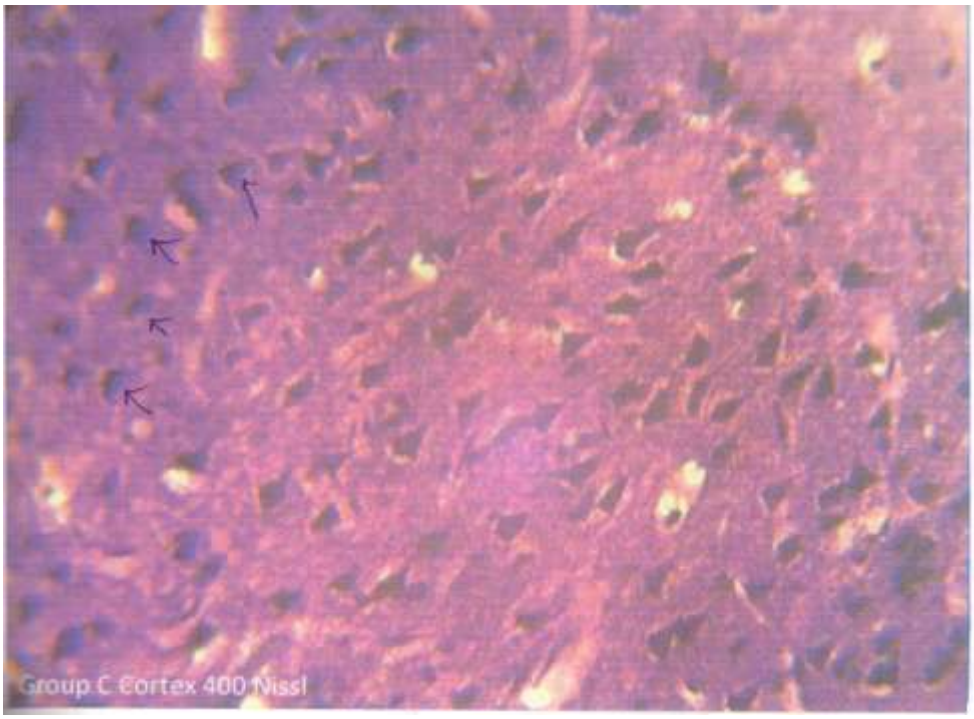

Plate 7: Photomicrograph of Group C Showing Few Cells Exhibiting Chromatolysis

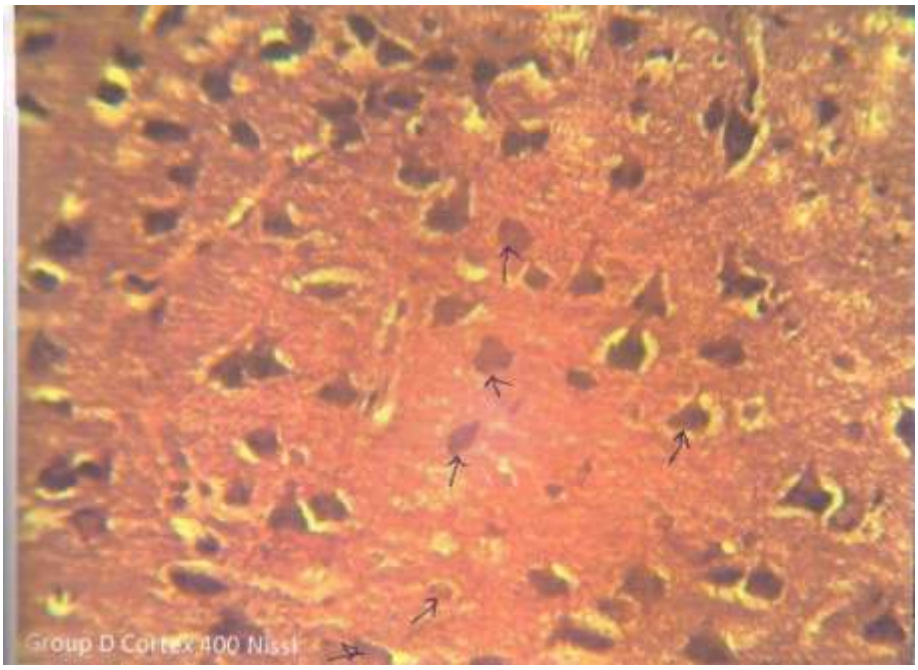

Plate 4: Photomicrograph of Group D Showing Numerous Cells Exhibiting Chromatolysis.

Discussion

a) **Body Weight**: The results of this study indicated that there was a significant ($P<0.05$) dose dependent decrease in weight of animals in the treated groups when compared with the control. Lead is a toxic metal that induces a wide range of behavioural, biochemical and physiological effects in humans (Goyer, 1990). Early symptoms of lead poisoning in adults are commonly non-specific and include depression, loss of appetite, intermittent abdominal pain, nausea, constipation and muscle pain (Merrill *et al.,* 2007). It therefore suggests that lead may have interfered with the normal physiology of the animals that resulted in loss of appetite and consequent loss in body weight.

b) **Histological Studies:** The cerebral cortex is a sheet of neural tissue that is outermost to the cerebrum of the mammalian brain. It plays a key role in memory, attention, perceptual awareness, thought, language and consciousness (Stephen, 2001). Cells of the cerebral cortex are related to the integration of sensory information and initiation of voluntary motor responses (Luiz and Jose, 2005). Cavanagh, 1984 stated that chemically induced neurodegeneration is usually characterized by different patterns of neuronal cell death, gliosis, swollen or destroyed axons or destruction of myelin sheath. These changes are usually preceded by changes on biochemical targets. In this study, hypertrophied cells, vacuolations around cells and cells at various stages of death were observed collectively. These histological changes imply cellular degeneration and early necrotic process in the cerebral cortex. The normal integrity of the cell body as well as the process is important for proper functioning of the CNS. When neuronal cell bodies are injured, various degeneration changes occur due to either obstruction in blood flow causing ischemia and hypoxia, crushing of new fibers and injection of toxic substance/chemical such as drugs (Abbas & Nelson, 2004). The synthesis and release of neurotransmitters may have also been impaired.

The brain is the most sensitive organ to lead exposure (Cecil *et al.,* 2008).The cerebral cortex of rats treated with lead acetate showed degenerative changes which may be as a result of the administration of lead acetate. The mechanism by which lead acetate caused these changes is unknown. However, lead interferes with the release of neurotransmitters (Dart *et al.*, 2004), and Xu *et al.,* (2009) also reported that the hippocampus of lead exposed rats showed structural damage such as irregular nuclei and denaturation of myelin.

c) **Histochemical Studies:** The neuron is one of the most complex cells in the body and since it is incapable of dividing after the first few days of life, loss of neurons are irreversible. A conspicuous feather in the perikaryon of large neurons is Nissl's granule which are rich in DNA and composed of stacks of rough endoplasmic reticulum and intervening group of free ribosomes (Macsween and Whaley, 1992) This makes Nissl's substance an important index in tracing neuronal population (John *et al.,* 1992). Chromatolysis is the migration of the Nissl's substance towards the periphery of the soma due to either trauma or due to other exogenous agents (Lowe, 1992; Snell, 2001).This usually result in loss of function or the loss of the protein synthesizing ability of the neurons and since proteins are the working molecules of cells, this may ultimately result in death of cells.

Conclusion

In this study, Nissl's substance was demonstrated in all the groups with few neuronal cells exhibiting chromatolysis in group C and more cells exhibiting chromatolysis in group D. This suggests that the effect of lead acetate is dose dependent. George *et al.,* (1998) reported that chemicals including drugs and toxins and oxygen lack cause alterations in the distribution patterns of Nissl's substance in the brain. The chromatolysis observed in groups C and D may be due to the toxic effect of lead acetate and this may affect protein synthesis in the neurons in the cerebal cortex and may consequently affect neuronal function in the cerebral cortex.

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