Original Research

Histopathological and Ultrastructural Changes of Brain Induced by Lead and Cadmium Alone and Combined Exposure in Male Wistar Rats

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Abstract

Lead and cadmium are most life threatening and well known central nervous system toxicants. This study was carried out to study histopathological and ultrastructural changes in brain induced by lead and cadmium alone and in combination in rats. Forty eight male albino Wistar rats were divided into 4 groups 12 rats in each group; group 1(control) was given deionized water, group 2 (lead group) was given water with lead acetate @ 30 mg/kg b.wt, group 3 (cadmium group) was given water with cadmium chloride @15 mg/kg b.wt, group 4 (combined group) was given water with both lead acetate@ 30 mg/kg b.wt and cadmium chloride @15 mg/kg b.wt for 28 days. The histopathological changes in brain revealed congested vessels, meningitis characterized by separation of meningial layer, hemorrhage underneath meningial layer and infiltration of MNCs and progressive degeneration of purkinje cells in cerebellum. Degenerative changes of nerve cell body and pockets of hemorrhage in cerebral cortex was noticed. The ultrastructural changes in brain showed degenerated neurons with thin myelin sheath, accumulation of granular electron dense material on sheath, microglial cells with abnormal nucleus and margination of chromatin, phagocytic cells with abnormal chromatin with swollen nucleus and increased perinuclear space.

Key words: Albino Wistar Rats, Histopathological and Ultrastructural Changes in Brain, Lead and Cadmium

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Introduction

Lead and cadmium are the two most abundant toxic metals in the environment. The common sources of lead and cadmium are diverse in nature including natural and anthropogenic processes such as combustion of coal and mineral oil, smelters, mining and alloy processing units and paint industries. Constantly increasing environmental pollutants due to increased urbanization, industrialization and through the scientific and technical advances have stimulated interest in the study of toxic substances and its consequences to biological system (Pandya *et al.*, 2012). Lead and cadmium are known potent neurotoxic

heavy metals which can induce oxidative stress and membrane disturbances in brain, rats exposed to lead and noticed vacuolation, degeneration of certain areas of cerebral cortex. Lead and cadmium enhances the production of free radicals in the brain of adult rats and interfere with the antioxidant defense system which in turn leads to alteration of the structural integrity of membrane lipids and secondarily affect the membrane bound enzymes such as Na^+ K^+- ATPase. (Kumar and Reddy *et al*., 2012). Cd depletes glutathione and protein-bound sulfhydryl groups, which lead to enhancement of reactive oxygen species generation (ROS) such as superoxide ion, hydroxyl radicals and hydrogen peroxide. Lead and cadmium are well known potent toxicants which cause tissue injury creating oxidative stress.

Material and Methods

Chemicals

Lead acetate and cadmium chloride were procured from Thermo Fisher Scientific India. Pvt. Ltd. Mumbai.

Experimental Animals

Adult male albino rats (*Wistar* strain*)* weighing 250-280g were procured from Sanzyme laboratories Ltd., Hyderabad. The experiment was carried out according to the guidelines and prior approval of the Institutional Animal Ethics Committee (IAEC) (No.18-2017 SA).

Experimental Design

A total of 48 male albino Wistar rats were randomly divided into 4 groups consisting of 12 in each group. Group 1(control) was given deionized water, group 2(lead group) was given water with lead acetate @30 mg/kg b.wt, group 3(cadmium group) was given water with cadmium chloride @15 mg/kg b.wt and group 4 (combined group) was given water with both lead acetate @ 30 mg/kg b.wt and cadmium chloride @15 mg/kg b.wt for 28 days respectively.

Methods

To study the histopathology and ultrastructural pathology, six rats from each group were sacrificed on 14th and 28th day of experimental period. Detailed necropsy was conducted and gross changes if any recorded. Pieces of brain were collected in fixative neutral buffer formalin (NBF) for histopathology. Samples were processed, sectioned (5µm), stained with Hematoxylin and Eosin (H&E) for as per the standard protocol given (Luna, 1968). The tissue samples of brain in 2.5% (percent) gluteraldehyde (PBS based EM grade) and processed for electron microscopic (EM) study as per the standard protocol (Bozzala and Russels, 1998).

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Results and Discussion

Section of brain of Group1 rats revealed normal histological architecture of cerebral cortex, cerebellum on 14th and 28th day of experimental period. Group 2 rats on 14th day of experiment revealed congested vessels (Fig. 1), meningitis characterized by separation of meningial layer, hemorrhage underneath meningial layer (Fig. 2) and infiltration of MNCs in cerebellum. On 28th day lesions noticed were congested vessels, degenerative changes of nerve cell body, and pockets of hemorrhage in cerebral cortex (Fig. 3). Where as in cerebellum in addition to meningitis, there was a progressive degeneration of Purkinje cells (Fig. 4).

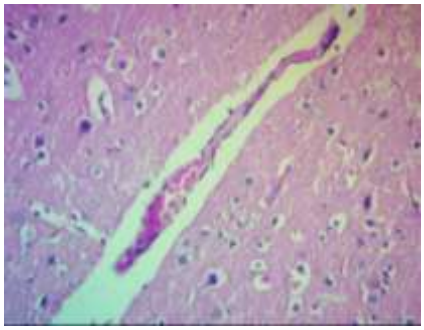

Fig. 1: Microphotograph of Group 2 section of brain (cerebrum) showing congestion of blood vessel on 14th day. H&E×400.

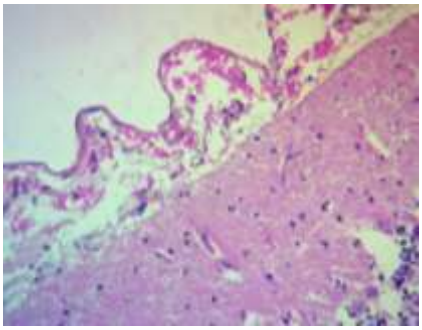

Fig. 2: Microphotograph of Group 2 section of brain (cerebellum) showing separation of meningeal layer and hemorrhage on14th day. H&E×400.

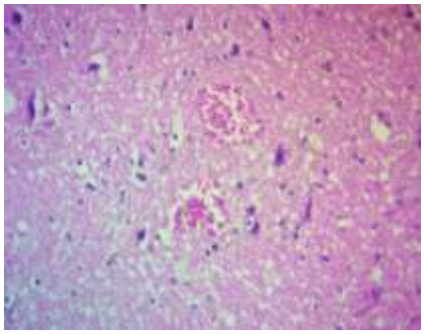

Fig.3: Microphotograph of Group 2 section of brain (cerebrum) showing pockets of hemorrhage on 28th day. H&E×400.

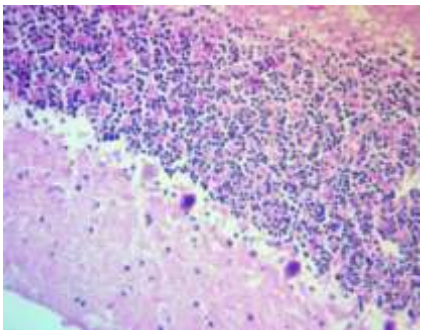

Fig. 4: Microphotograph of Group 2 section of brain (cerebellum) showing progressive degeneration of purkinje cells on 28th day. H&E×400.

The section of brain from Group 3 revealed congested vessels (Fig. 5), perivascular cuffing in cerebral cortex (Fig. 6) and multi focal areas of gliosis (Fig. 7). In cerebellum also gliosis was noticed. On 28th day in addition to changes noticed on 14th day there was marked lymphocytic infiltration in brain. Brain section of Group 4 rats on 14th day revealed marked congested vessels (Fig. 8), nerve cell bodies nearby blood vessels in pattern of degeneration and perivascular cuffing (Fig. 9) in cerebral cortex. Cerebellum showed progressive degeneration of Purkinje cells. On 28th day similar changes were as that of 14th day but

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progressive in nature. Separation of meningial layer and hemorrhage underneath meningial layer more prominently noticed in cerebral cortex (Fig. 10) along with degeneration of Purkinje cells (Fig. 11).

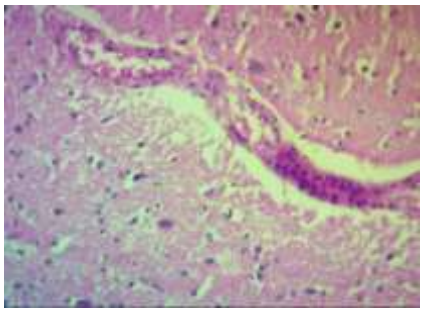

Fig. 5: Microphotograph of Group 3 brain (cerebellum) section showing congested vessels on 14th day. H&E×400

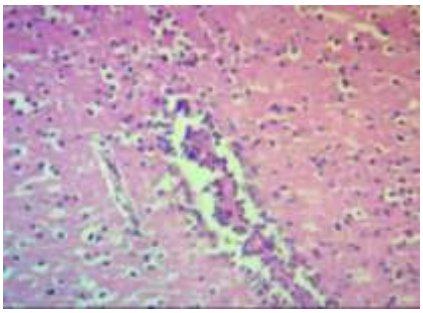

Fig. 6: Microphotograph of Group 3 brain (cerebrum) section showing mild perivascular cuffing on 14th day. H&E×400.

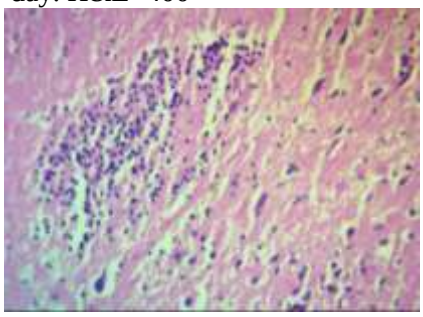

Fig. 7: Microphotograph of Group 3 brain (cerebellum) section showing gliosis on 28th day. H&E×400.

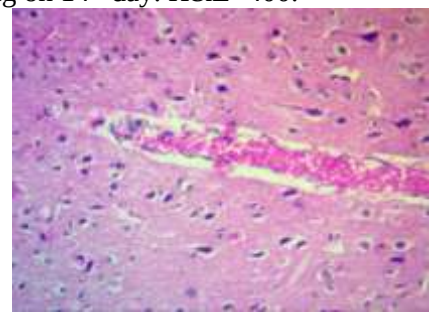

Fig. 8: Microphotograph of Group 4 brain (cerebrum) section showing severe congested blood vessel on 14th day. H&E×400.

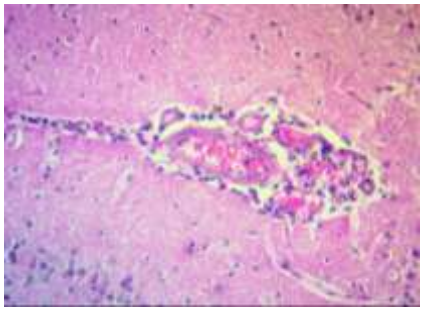

Fig. 9: Microphotograph of Group 4 brain (cerebrum) section showing severe congested blood vessel and perivascular cuffing on14th day. H&E×400.

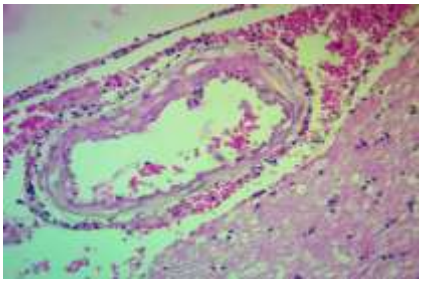

Fig. 10: Microphotograph of Group 4 brain (cerebrum) section showing meningitis characterized by infiltration of MNCs, severe hemorrhage and separation of meningeal layer on 28th day.H&E×400.

EM section of brain of Group 2 rats showed group of degenerated neurons showed demyelination along with accumulation of granular electron dense material (Fig. 12). Microglial cells with abnormal nucleus and margination of chromatin was observed (Fig. 13, 14). Group 3 rats showed degenerated neurons with

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thin myelin sheath, granular ECM, accumulation of granular electron dense material on sheath (Fig. 15, 16).

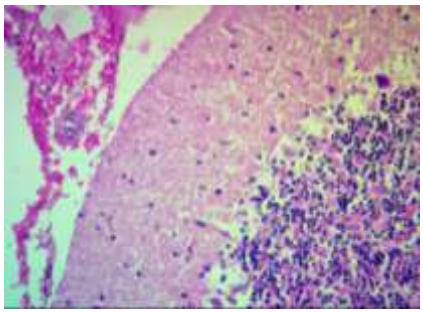

Fig. 11: Microphotograph of Group 4 brain (cerebellum) section showing loss of Purkinje cells on 28th day.H&E×400.

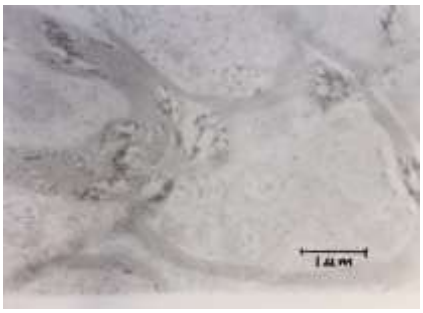

Fig. 12: Transmission electron micrograph of brain (cerebrum) showing thin myelin sheath with accumulation of electron dense granular deposits on myelin layer. UA & LC 19300x (Group 2, day 28).

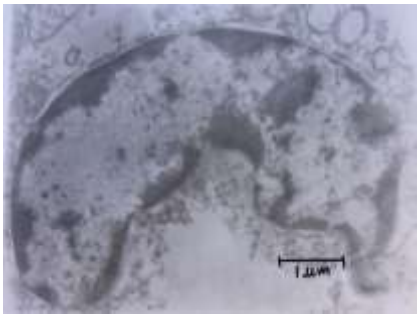

Fig. 13: Transmission electron micrograph of brain (cerebrum) showing Microglial cell with increased perinuclear space, swollen nucleus with abnormal chromatin with severe margination of chromatin material UA & LC 19300x (Group 2, day 28).

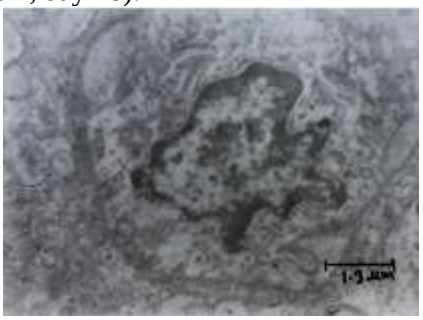

Fig. 14: Transmission electron micrograph of brain (cerebrum) showing microglial cell with abnormal nucleus and margination of chromatin material. UA & LC15440x (Group 2, day 28).

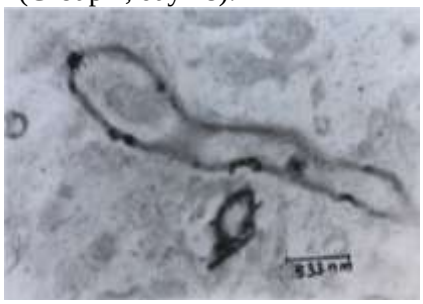

Fig. 15: Transmission electron micrograph of brain (cerebrum) showing degeneration of neuron, thin myelin layer and electron dense deposits over the surface of demyelinated area. UA & LC 23160x (Group 3, day 28).

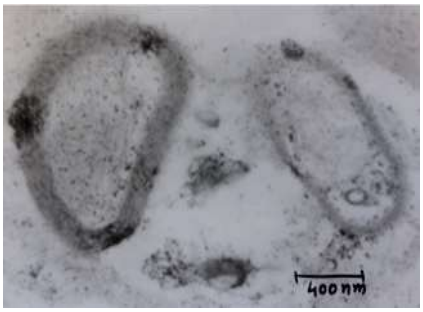

Fig. 16: Transmission electron micrograph of brain (cerebrum) showing granular extra cellular matrix and electron dense deposition on myelin layer. UA & LC 48250x (Group 3, day 28).

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Group 4 rats showed distorted nucleus with prominent perineuronal vacuolation (Fig. 17). Increased perineuronal space, electron dense granular nurolemma of degenerated neuron, with pyknotic nucleus, electron dense chromatin and clumped and severe margination of chromatin material was observed (Fig. 18, 19). In some other sections altered nucleoplasm and cytoplasm ratio was noticed. In some other sections glial cell with increased perineuronal space, swollen nucleus and electron dense chromatin, mild to severe perinuclear vacuolation in the vicinity of degenerated glial cell were noticed (Fig. 20).

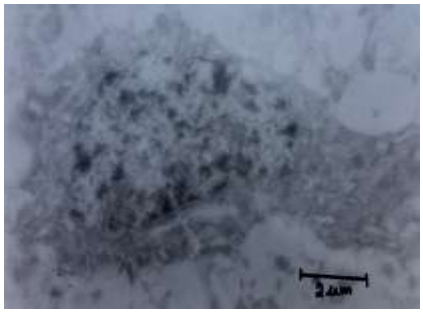

Fig. 17: Transmission electron micrograph of brain (cerebrum) showing distorted nucleus with enlarged nucleus and prominent perineuronal vesiculation. UA & LC 9650x (Group 4, day 28).

Fig. 18: Transmission electron micrograph of brain (cerebrum) showing margination of chromatin material in nucleus, electron dense material in granular neuroplasma of degenerated neuron. UA & LC 11580x (Group 4, day 28).

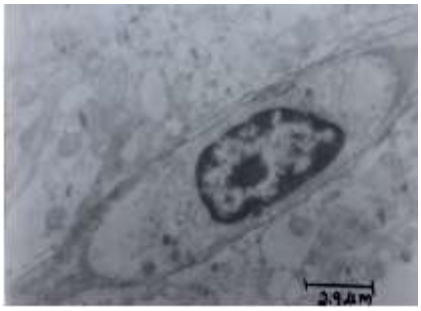

Fig. 19: Transmission electron micrograph of brain (cerebrum) showing degenerated neuron with pyknotic nucleus, electron dense chromatin and clumped and severe margination of chromatin material. UA & LC 6755x (Group 4, day 28).

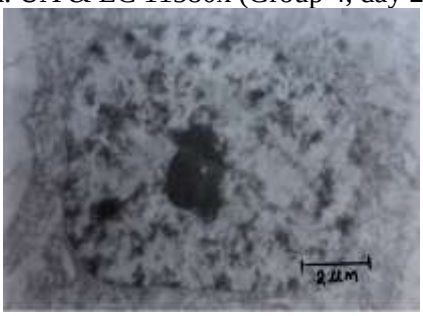

Fig. 20: Transmission electron micrograph of brain (cerebrum) showing glial cell with increased perineuronal space, swollen nucleus and electron dense chromatin. UA & LC 9650x (Group 4, day 28).

In present study brain of Group 2 rats on 14th day revealed congested vessels, nerve cell degeneration nearby blood vessels, meningitis characterized by separation meningial layer, hemorrhage underneath meningial layer, infiltration of MNCs in cerebral cortex which were in agreement with Kumar and Reddy *et al*., 2012 and AI-Naimi *et al*. (2011). Where as in cerebellum in addition to meningitis, there was a progressive degeneration of Purkinje cells which was supported by Sidhu & Nehru (2004). On 28th day severe congested vessels, degenerative changes of nerve cell body and pockets of hemorrhage in cerebral

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cortex was noticed. Metal toxicity or lead toxicity affects the normal histological structure of the brain and causes disturbances in the normal functions as opined by Clasen *et al*. (1974).

Section of brain of Group 3 rats, revealed congested vessels, multi focal areas of gliosis, perivascular cuffing and hemorrhage in cerebral cortex and gliosis in cerebellum, these lesions were in consistent with the findings of Yoshida, 2001. On 28th day in addition to changes noticed on 14th day there was marked lymphocytic infiltration in brain. Reason for hemorrhage in brain was explained as the administration of cadmium initially affects the integrity and permeability of the vascular endothelium which led to extensive hemorrhages in the cerebral and cerebellar cortices in rats which were exposed to cadmium (Gabbiani *et al*., 1967; Wong and Klaassen (1982). Brain section of Group 4 rats on 14th day revealed marked congestion of vessels, nerve cell bodies degeneration, gliosis in cerebral cortex. Cerebellum showed progressive degeneration of purkinje cells. These lesions were related to investigation of Randa *et al*. (2012). On 28th day lesions were progressive in nature. Separation of meningial layer, hemorrhage underneath meningial layer more prominently noticed in cerebral cortex. All most similar changes were reported by M´endez-Armenta *et al*. (2001).

EM section of brain of Group 2 rats showed group of degenerated neurons with thin myelin sheath, accumulation of electron dense granular material on sheath, microglial cells with margination of chromatin with swollen nucleus and increased perinuclear space. Group 3 rats showed degenerated neurons with thin myelin sheath, granular ECM, accumulation of granular electron dense material on sheath. Group 4 rats showed distorted nucleus with prominent perineuronal vacuolation, increased perineuronal space, swollen nucleus and electron dense granular nurolemma of degenerated neuron. In some sections there was altered nucleoplasm and cytoplasm ratio, mild to severe perinuclear vacuolation in the vicinity of degenerated glial cell along with the changes noticed in Group 2 and Group 3. These changes were supported by Zhang *et al*. (2009) and Deveci (2006). These ultrastructural changes may be due to lead and cadmium induced high production of free radicals which in turn causing lipid peroxidation, mitochondrial disruption and cell death. The primary effects of lead on brain thought to be damage to the nervous system, microvasculature, demyelination, inhibition of axonal activity Rodier (1995) and Schmitt *et al*. (1996).

Conclusion

In conclusion, this study shows that lead and cadmium are the potent toxicant which can cause damage of tissue architecture and subcellular changes. Lead and cadmium administered in combination has a potentiated effect. It is also concluded that lead and cadmium are the potent inducers of oxidative damage of brain. The present study therefore provides investigatory evidence of supporting lead and cadmium toxicity in albino Wistar rats.

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