Effect of Cadmium Exposure on the Structure of the Cerebellar Vermis of Growing Male Albino Rat

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ABSTRACT: Contamination of the environment with cadmium may impact human health through the persistent exposure to small doses over a long period of time. The present work was planned with the aim to study the histological changes that might occur in the vermis of the cerebellum in growing male albino rats following cadmium exposure. Sixty growing male albino rats, weighing 47 - 59 grams, were utilized in this study. The animals were divided into 3 groups; each of 20 rats: animals of group I served as control, animals of group II received 2 ml of cadmium chloride 0.4 mg / Kg / day solution with drinking water for 8 weeks and those of group III received the same dose by the same route for 12 weeks. At the end of the experiment the rats were sacrificed. The vermis of the cerebellum was dissected and processed for paraffin sections and stained, for light microscopic examination, by haematoxylin and eosin, cresyl violet acetate stain, luxol fast blue stain and immunohistochemical labeling of astrocytes using anti-glia fibrillary acidic protein. Ultrathin sections were prepared for electron microscopic examination. This study revealed changes in the vermis of the cerebellum, after exposure to cadmium, including vascular, neuronal, white matter and astrocytic changes. Vascular changes appeared in the form of congestion and perivascular oedema as well as dilatation and distortion of the capillaries with destruction of their endothelial lining. Some Purkinje cells showed degeneration, distortion with small nuclei, abnormal arrangement in many layers, loss of nuclear cytoplasmic differentiation, deformity of nuclei, increase of mitochondria, proliferation of rough endoplasmic reticulum and excessive primary and secondary lysosomes. Granular cells became loose with less packing with decrease of mitochondria and ribosomes, shrunken deformed nuclei, surrounded by macrophages containing electron dense material and also surrounded by distorted nerve fibers. The white matter showed pleomorphic destruction. Proliferation and increase in the astrocytes with increase of their branches. All these cerebellar changes were more prominent with prolongation of the period of cadmium exposure. The present study demonstrated that continuous oral administration of cadmium for 8 weeks and 12 weeks has hazardous toxic effects on the structure of the vermis of the cerebellum in growing albino rats. It is recommended to avoid as much as possible, the use of cadmium and its compounds in different industries to prevent contamination of air, soil, water and food with this major environmental toxin.

Keywords: Animal; Cadmium; Cerebellar vermis; Neurotoxicity; Histology

INTRODUCTION

The effect of cadmium on the various regions of the central nervous system of albino rats has been conventionally described by many authors: Olson, et al (2009), Draff and Peters (2011), Graef (1998), and Flower (2006), but the information available from the literature about its effect on the vermis of the cerebellum is still lacking and controversial.

There has been a major resurgence of interest in cadmium toxicity with numerous publications in both the scientific and lay press, Wolfe and Molinoff, (2012). Comprehensive reviews have recently been published on the

Clearly, cadmium is a major environmental toxin due to its presence in air, water, food and soil Graef, (1998) Cadmium and its compounds may be used in the manufacture of paints, coatings, rubber, insecticides, colored printing inks and glass, Collier, et al (2010) and Ernhart, et al (2001) . Rogers and Davis (2013) reported that human beings have always been exposed to cadmium but its amount is substantially increased due to industrial production. Koch and Berrera, (2006) mentioned that other major cadmium sources include metal smelters, mining, cement production and garbage burden.

Beal, et al (2011) observed that high doses of cadmium were toxic in rats as well as in humans and it acted mainly on the endothelial cells of the blood vessels of the central nervous system. This caused an increase in capillary permeability to blood cells and proteins with vasogenic oedema, Kumar (2008). On the other hand, Koch and Berrera, (2006) found that low doses of cadmium did not increase capillary permeability to blood cells and proteins, but it could affect the normal function of endothelial cells by affecting the transport of some important metabolites. Lund, et al (2004) noticed haemorrhage in the cerebellum, spinal cord and cerebral hemispheres but not in pons and medulla. De Perio, et al (2010) recorded that rats administered cadmium had the highest concentration of cadmium in the cerebellum at all age and dose groups. This made the cerebellum one of the most vulnerable areas of cadmium neurotoxicity, Scheibel, et al (2008).

On the contrary, Rogers and Davis (2013) denied the presence of any cadmium toxicity on the cerebellum of albino rats. Although most of the available researches dealt with the biochemical effects of cadmium on the different organs; however few of them described the histological changes induced by cadmium on these organs. So the aim of this study is to evaluate the possible deleterious effects of cadmium on the histology and ultra-structure of the cerebellar vermis in growing male albino rats exposed to small dose of cadmium that would not alter the growth and body weight of these rats.

MATERIAL AND METHODS

A total of 60 growing male albino rats, weighing 47 - 59 grams, were utilized in this study. At the age of 3 weeks, the sex could be easily determined and the growing rats were selected. They were kept in metal cages under standard condition and were fed ad libitum on powdered laboratory chow and tap water. In this study, cadmium chloride crystals 100%, obtained from El-Nasr Chemical Company, Jeda, KSA were used and dissolved in distilled water at concentration 0.4%, Scheibel, et al (2008). The animals were divided into 3 groups; each of 20 rats: Group I (Control group), divided into two equal subgroups: Subgroup Ia drank water free from cadmium for 8 weeks and subgroup Ib drank water free from cadmium for 12 weeks, Group II, received 2 ml of cadmium chloride 0.4 mg / Kg / day solution with drinking water daily for 8 weeks , Peters, et al (1999), while Group III, administered 2 ml of cadmium chloride 0.4 mg / Kg / day solution with drinking water daily for 12 weeks , McConnel, (2004).

At the end of the experimental periods, the animals were sacrificed. This was followed by fixation of the cerebellum in situ by perfusion fixation technique, Kale, et al (2005). 10% formal saline was used as a fixative for specimens processed for light microscopy, while a mixture containing 2.5% glutaraldehyde and 1.5% para-formaldehyde in 0.1 mol/L phosphate buffer (pH 7.2) was used for fixation of specimens processed for electron microscopy.

A. For light microscopy, after perfusion with formal saline, the skull vault was opened and the skull was put as a whole in the same fixative for one week. Then the vermis of the cerebellum was dissected and processed for paraffin sections subjected to the following histological and immunohistochemical techniques. Haematoxylin and eosin for the general structure, Drury and Wallington, (1996) Cresyl violet acetate staining for Nissl granules, Bowling, (2005) Luxol fast blue staining for the myelin, Haldimann, et al (2007)

Glia fibrillary acidic protein (GFAP) staining using rabbit anti-glial fibrillary acidic protein antibodies delivered from Sigma laboratory to examine the astrocytes. The positive results were indicated by brown coloration of the cell membrane and cytoplasm of the astrocytes. This stain is specific for the intermediated filaments fibrillary acidic protein found in astrocytes and is not found in nerve cells and even other types of glial cells as microglia or oligodendroglia , Sternberger, (2006).

B. For electron microscopy, after fixation with paraformaldehyde-glutaraldehyde mixture, the skull was obtained then the whole cerebellum was obtained and put in the same fixative for 6 hours at 4°C. The selected regions from the vermis of the cerebellum were taken and semithin sections were cut at 1 µm thickness by
ultramicrotome, stained with toluidine blue and examined by light microscope to detect the areas of interest. Ultrathin sections (50 nm) were prepared using the same ultramicrotome and stained with uranyl acetate for 20 minutes and lead citrate for 10 minutes, Hayat, (1996) . These ultrathin sections were examined by transmission electron microscope and photographed under different magnifications for detection of ultrastructural pathologic changes.

RESULTS

Group I (Control group): Light microscopic examination of the vermis of the cerebellum of the control albino rats revealed that the cerebellum was formed of outer cortex of grey matter overlying the central medullary mass of white matter (Figure. 1). The cerebellar cortex was found to be composed of three well defined layers. These layers were the outer molecular layer, the inner granular layer and the Purkinje layer in between the previous two layers (Figure. 1). The molecular layer contained relatively few cells and showed an outer stellate cell and an inner basket cells located near the Purkinje layer (Figure. 2). The Purkinje cells appeared large, flask shaped, uniformly arranged in a single layer of cells along the outer surface of the granular layer (Figure. 2). The Purkinje cells had relative fine axons extending deep through the granular layer and an extensively branching dendritic system that arborized into the outer molecular layer (Figure. 2). Each Purkinje cell had a clear vesicular nucleus with deeply stained nucleolus (Figures. 2 & 3). The deep granular layer was composed of numerous, small, closely packed and deeply stained neurons whose axons passed outwards to the molecular layer (Figure. 2). The cells in the granular layer had excessive Nissl granules while the Purkinje cells had irregular scanty Nissl granules (Figure. 3). The normal myelinated nerve fibers of the white matter were homogenous deeply stained and branching (Figure. 4). With immunohistochemical staining of the astrocytes using glial fibrillary acidic protein antibodies revealed uniform distribution of the astrocytes that were small in size with few processes (Figure. 5).

Electron microscopic examination of ultrathin sections of the cerebellar vermis of control rats demonstrated that each Purkinje cell had a large central oval nucleus surrounded by cytoplasm containing healthy cell organelles as mitochondria, free ribosomes, rough endoplasmic reticulum and Golgi apparatus (Figure. 6). On the other hand, the granular cell layer revealed densely packed granular cells. Each granular cell had a central, large, oval nucleus with peripheral clumped chromatin and this nucleus was surrounded by a thin rim of cytoplasm containing few mitochondria and free ribosomes (Figure. 7).

Group II: Light microscopic examination of the cerebellar vermis of the rats in this group revealed oedema in molecular, Purkinje and granular layers with separation of their cells (Figures. 8- 10). Congestion of blood vessels and perivascular oedema appeared in the molecular layer (Figure. 8). Glial proliferation was also noticed in the molecular layer (Figure. 9). Some Purkinje cells were degenerated deeply stained with loss of their nuclei (Figure. 8). Many Purkinje cells were arranged in many layers intermingling with granular cells (Figure. 9) or showed pleomorphism with variable amount of Nissl granules (Figure. 10). The granular cells became loose and less packing (Figures. 9 & 10). Mild pleomorphic destruction of myelinated fibers in the white matter was observed (Figure. 11). Mild proliferation and increase in the astrocytes and their branches were also noticed (Figure. 12).

Electron microscopic study of the vermis of the cerebellum of albino rats in this group showed mild changes in the Purkinje and granular cells. Purkinje cells had large irregular deformed nuclei, increase in number of mitochondria and free ribosomes, proliferation of rough endoplasmic reticulum and decrease in Golgi apparatus (Figure. 13). The granular cells demonstrated normal large central oval nucleus and decreased number of mitochondria and free ribosomes (Figure. 14). These cells were surrounded by normal nerve fibers and microglial cells which contained electron-dense material surrounded by electron-lucent mass (Figure. 14).

Group III: In this group of cadmium exposed rats, light microscopy showed marked oedema and vacuolations in all layer of cerebellar vermis (Figures. 15 & 16). The Purkinje cells appeared degenerated, distorted and small in size with pyknotic nuclei (Figures. 15 & 16). There were decreased cellular density and packing in the granular cell layer (Figures. 15 & 16). There were vascular changes in the molecular layer in the form of dilatation and distortion of the capillaries with destruction of their endothelial lining whose debris was found inside the lumen (Figures. 15 & 16). In some specimens, abnormal arrangement of Purkinje cells in more than one layer intermingling with granular cells (Figure. 16). Some of these cells lost their nuclear-cytoplasmic differentiation (Figure. 16). Nissl granules increased in the Purkinje cells and decreased in the granular cells (Figure. Kumar, T.A. (2008)). Marked pleomorphic destruction of the myelinated nerve fibers in the white matter was noticed in the cerebellum of this group (Figure. 18). There was marked increase in the thickness and branching of astrocytic processes with increased intensity of immunostaining due to astrocytic proliferation (Figure. 19).

Electron microscopic examination of the vermis of the cerebellum from this group revealed marked changes in the Purkinje cells which had irregular deformed nuclei (Figure. 20). These cells demonstrated marked increase in the mitochondria and proliferation of the rough endoplasmic reticulum with appearance of excessive primary and
secondary lysosomes (Figure 20). On the other hand, the granular cells showed shrunken, deformed and irregular nuclei which were surrounded by electron dense cytoplasm (Figure 21). The granular cells were surrounded by swollen astrocytic processes and macrophage cells which phagocyted electron dense material (Figure 21). In addition, the nerve fibers, surrounding the granular cells, were distorted in their contour (Figure 21).

DISCUSSION

Cadmium is an environmental toxin present in air, water, food and soil as well as it is widely used in the manufacture of rubber, glass, paints, coatings and colored printing inks, Graef, (1998), and Collier, et al (2010). The neurotoxicity of cadmium has been recently recognized by many authors. Ernhart, et al (2001). Exposure to high cadmium level in humans is rare, resulting in serious central nervous system damage including encephalopathy, peripheral neuropathy and hemorrhage which may affect the cerebrum and/or the cerebellum, Jackson, (2008), and Watenaux, et al (2009). On the other hand, subclinical cadmium poisoning from chronic low level cadmium exposure remains a major health problem especially in industrial workers, Sidman and Leviton (2005). The cadmium neurotoxicity was chosen as the target for this study as it is of recent interest and one of the most dangerous heavy metals that can insult the environment. In the current study male albino rats were used as a mammalian model for studying the possible neurotoxicity of cadmium, as they are available, easy in handling, sensitive to cadmium toxicity and to avoid hormonal and physiological changes in female rats, Globus, et al (1999). The brain is a major cadmium depot in the body and has no known mechanism for its elimination, Herbert and Globas, (2005). Minneman, et al (2004), mentioned that the distribution of cadmium in the cerebellum was more than the cerebrum and the highest accumulation was noticed in the vermis of the cerebellum. These observations directed the decision to the cerebellar vermis to be the target site for this study.

In the present work, cadmium chloride in low dose (2 ml of cadmium chloride 0.4 mg / Kg / day) was used. Since many authors suggested that the effect of cadmium on the central nervous system could be attributed to cadmium-induced malnutrition and not due to the direct influence of environmental cadmium burden on the nervous tissues, therefore the dose used in this study was adjusted not to alter the body weight or induce growth retardation and malnutrition Graef, (1998) and Lund, et al (2004). In addition, this low dose of cadmium was adjusted to avoid animal death and this dose is also similar to the cadmium environmental level in water, Scheibel, et al (2008). Lauder and McCarthy (2006) recorded that in acute cadmium intoxication, the lethal dose causing death to 50% of rats (LD$_{50}$) was 10 ml of cadmium chloride 6.5 mg / Kg.

The route of administration in the present study was via drinking water because it is the commonest route for cadmium intoxication, Peters, et al (1999), and McConnel, (2004). Adjustment of blood cadmium level was not considered because the aim of this work was to expose the rats to low doses of cadmium which are unnecessary to be constant to simulate the environmental exposure pattern.

This research revealed variable vascular changes in the vermis of the cerebellum following cadmium administration. These changes appeared in the form of oedema and vacuolations in all layers of the cerebellum with separation of their cells, and congestion of blood vessels with perivascular oedema. Dilatation and distortion of the capillaries with destruction of their epithelial lining were also detected. These vascular changes were more severe with prolongation of the period of exposure to cadmium. These observations are in agreement with the results of, Wolfe and Molinoff, (2012) who added that alteration in the blood brain barrier permeability led to increase plasma protein content in watery transudate causing oedema that might be followed by haemorrhage in more advanced cases. Raki, et al (2003), using radiolabeled cadmium, reported that cadmium deposition was mainly within the endothelial cytoplasm and concluded that the primary lesion in cadmium toxicity was vascular injury. On the other hand, no haemorrhage could be detected in the present work. This could be attributed to the low dose of cadmium exposure used. On the contrary, Kale, (2005) found petechial haemorrhages in all parts of the cerebellum, seven days following initiation of cadmium administration, which increased in size and number to the extent of a total haemorrhagic cerebellum.

The current study revealed gial proliferation in the molecular layer and some Purkinje cells were degenerated, deeply stained with loss of their nuclei. Many Purkinje cells were arranged in many layers intermingling with granular cells and some showed pleomorphism with variable Nissl granules. In some specimens, Purkinje cells appeared distorted, smaller with pyknotic nuclei arranged in many layers intermingling with granular cells or loss of their nuclear-cytoplasmic differentiation. These results are in consistency with those of Fairhall, et al (2012). The latter author added that Purkinje cells were more susceptible to the neurotoxic effect of cadmium which might be dependent on the chemical receptors within these cells. The abnormal arrangement of these cells in more than one layer, noticed in this study, might indicate retardation of development and delay in their arrangement into single layer.
The present electron microscopic investigation showed early mild changes in the ultrastructures of Purkinje cells in the form of irregular nuclei, increased mitochondria, proliferation of the rough endoplasmic reticulum and an increase in the free ribosomes. With prolonged exposure to cadmium, the severity of the previous changes in Purkinje cells had increased with the appearance of primary and secondary lysosomes. These observations are close to those recorded by Raki, et al (2003). In addition, Wallington, (2011) reported destruction of nuclei and mitochondria with shrinkage of Purkinje cells. Moreover, Marc et al (2009) suggested that the primary effect of cadmium on the cerebellum was in the Purkinje cells due to tendency of heavy metals to accumulate within their cytoplasm. On the other hand, Jackson, (2008) demonstrated that the effect of cadmium on the blood-brain barrier might decrease nutrition and produce hypoxia which affected the morphology of Purkinje cells and might lead to degeneration or even necrosis of these cells. On the contrary, Koch and Berrera, (2006) denied marked abnormalities in the Purkinje cells after the intake of cadmium for eight weeks and suggested that the neurotoxic effect of cadmium was mainly on the granular cells. Wang, et al (2005) recorded that appearance of lysosomes could be considered as early stage of destruction of Purkinje cells.

Mild changes in the granular cells of the cerebellum were detected in this experiment following cadmium administration. These cells appeared loose, less packed with decrease density and also there was decrease of Nissl granules. Ultrastructural examination of these granular cells showed a decrease in each of the mitochondria and free ribosomes, only in case of exposure to cadmium for 8 weeks. On the other hand, administration of cadmium for a longer period (12 weeks) resulted in severe neuronal changes in the form of shrunken, irregular, deformed nuclei, surrounded by electron dense cytoplasm and distortion of nerve fibers surrounding these cells. These results are in agreement with the reports of Rogers and Davis, (2013) who demonstrated that the effect of cadmium on the cerebellar neurons of growing albino rats was retardation of their development and causing necrosis to mitotically active precursor cells which were considered the most susceptible to cadmium toxicity. These structural changes associated with delayed maturation of cerebellar neurons in growing albino rats could be considered a reasonable etiology of some functional alteration observed in the experimental animals and human children exposed to cadmium as delayed acquisition of some mental abilities and delayed behavioural and emotional maturity lagging behind children at the same age, Sidman and Leviton (2005).

The present work demonstrated that the destructive effect of cadmium on the myelinated nerve fibers in the white matter of the cerebellum of albino rats was pleomorphic. This effect was more severe with prolongation of the period of cadmium administration. Moreover, with prolonged exposure to cadmium, the nerve fibers surrounding the granular cells were distorted in their contour. These observations are in accordance with the reports of Kumar, (2008) who also added that cadmium toxicity on the myelinated nerve fibers was dose-dependent and described similar toxic effects in the white matter of hippocampus and hypothalamus. The hypomyelination observed in cadmium exposed growing albino rats could be explained in the light of the results of, Sidman and Leviton (2005) who suggested that these changes were secondary to retarded neuronal growth and maturation in growing rats. In addition, Krieg and Berry, (2009) recorded that inhibition and somewhat retarded myelination in growing rats might be due to a reduction in the number of myelin generating glial cells as well as the restricted capacity of these cells. In contrast, Rogers and Davis (2013) found that the investment of myelin relative to the axons was normal in cadmium intoxicated rats. Moreover, Minneman, et al (2004) mentioned that without concomitant growth retardation, postnatal cadmium exposure did not cause hypomyelination or destruction of nerve fibers in the rat brain.

Proliferation and increase in the astrocytes with increased intensity of immune-staining and increase of their branches, following cadmium administration, were detected in this research. These morphological alterations could be considered as a hyperactivity of astrocytes in a trial to accommodate the toxic metal by forming glutathione to bind the metal in an attempt to protect neurons from its hazardous effect.

The use of immunohistochemistry in this study was based on the suggestion of Sternberger, (2006) who proved that glial fibrillary acidic protein (GFAP) was considered as a sensitive and specific indicator for cadmium neurotoxicity. Different types of CNS damage were found to induce astrocytic response Flower, (2006). This suggested that cadmium exposure could be considered as a powerful cause of CNS damage as detected by the high astrocytic proliferative response after cadmium administration, Allen, et al (2006). Lund, et al (2004) recorded that astrocytes had great affinity to cadmium and could be able to protect the neuronal cells from its potential hazards but carrying a great danger as it had no known mechanism for its chelation so carrying a very dangerous depot of cadmium that might destroy the neurons if these astrocytes reached a level of cell death with liberation of their cadmium in the brain. Draf and Peters (2011) concluded that, astrocytes were considered the primary target for cadmium-intoxication even before any neuronal affection. These cells played a major role in neurotransmitter uptake and metabolism, neurotransmitter receptor expression, neurotrophic factor-secretion and secretion of extracellular matrix protein, Watenaux, et al (2009).
The present electron microscopic investigation revealed macrophage cells which contained electron dense bodies. This finding is in consistency with, Wolfe and Molinoff, (2012) who suggested that these bodies were either phagocytosed material especially red blood cells or degradation products of early degenerative changes as in brain aging or as in Alzheimer's disease. These electron dense bodies were present in many aging cerebellar processes and called corpora amylacea, Sidman and Leviton (2005). The latter authors concluded that cadmium might induce premature aging process.

It conclusion, the current investigation has shown that exposure to cadmium for 8 or 12 weeks initiates extensive changes in the vermis of the cerebellum of growing male albino rats. The toxicity of cadmium affects all structural elements of the cerebellum including neuronal, astrocytic and vascular changes as well as involvement of white matter. All these changes become more marked with prolongation of cadmium exposure. Great efforts should be carried out to control environmental pollution with cadmium as it forms a real threatening element for the mentality and behavior of the children who are considered the most vulnerable group for this metal. The mental health of our children is very precious so they should be the focus of our plans for a clean future free from such hazardous heavy metal.

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Figure 1. A photomicrograph of a paramedian section in the vermis of the cerebellum of a control rat showing the different layers of the cerebellar cortex which are molecular layer (M), Purkinje layer (arrow heads) and granular layer (G), as well as the central medulla which is formed of white matter (W). (Hx. & E X200)

Figure 2. A photomicrograph of a paramedian section in the vermis of the cerebellum of a control rat showing the molecular layer (M) which includes the outer satellite cells (S) and the inner basket cells (B) located near the Purkinje cells. The Purkinje cells (P) are seen large flask shaped, uniformly arranged, with a large vesicular nuclei and deeply stained nucleoli. The Purkinje cells have a branching dendritic system (arrow heads) and small axon (arrow). The granular layer is composed of closely packed deeply stained numerous small neurons (G) and axons (A) which pass outwards to the molecular layer. (Hx & E X400)
Figure 3. A photomicrograph of a paramedian section in the vermis of the cerebellum of a control rat showing the cells of the Purkinje layer (P) which are pale stained and the cells of the granular layer (G) which are deeply stained with excessive Nissl granules. The Purkinje cells have large vesicular nuclei (arrow), deeply stained nucleoli (arrow head) and irregular scanty Nissl granules.(Cresyl violet  X1000)

Figure 4. A photomicrograph of a paramedian section in the vermis of the cerebellum of a control rat showing the branching homogenous myelinated white matter (W) of the cerebellum.(Luxol fast blue  X40)

Figure 5. A photomicrograph of a paramedian section in the vermis of the cerebellum of a control rat showing the molecular layer with a uniform immunohistochemical reaction in astrocytes (arrows) which are small in size with few branches (arrow heads).(Immunohistochemical stain for GFAP  X1000)
Figure 6. An electronmicrograph in the vermis of the cerebellum of a control rat showing a Purkinje cell with a large central oval nucleus (N) which is surrounded by cytoplasm containing mitochondria (M), free ribosomes(R), rough endoplasmic reticulum(ER) and Golgi apparatus(G). (Uranyl acetate and lead citrate X5000)

Figure 7. An electronmicrograph in the vermis of the cerebellum of a control rat showing granular cells (G) which are crowded in groups. These cells have central large oval nuclei (N) with peripheral clumped chromatin (arrows), which are surrounded by thin rim of cytoplasm. This cytoplasm contains mitochondria (M) and free ribosomes (R). (Uranyl acetate and lead citrate X5000)

Figure 8. A photomicrograph of a paramedian section in the vermis of the cerebellum of a rat from group II showing oedema (arrows) in the molecular (M) Purkinje (P) and granular (G) layers. Purkinje cells are degenerated, deeply stained with destruction and loss of their nuclei. Congestion of blood vessels and perivascular oedema (arrow head) appear in the molecular layer. (Hx & E X 400)
Figure 9. A photomicrograph of a paramedian section in the vermis of the cerebellum of a rat from group II showing mild oedema (arrows) of all layers with separation of their cells. There is glial proliferation (arrow heads) in the molecular layer (M). Purkinje cells (P) are arranged in many layers intermingling with granular cells (G) which are less packing than the control group. (Hx & E X400)

Figure 10. A photomicrograph of a paramedian section in the vermis of the cerebellum of a rat from group II showing oedema (arrows) of all layers with marked wide separation of their cells. Purkinje cells (P) reveal pleomorphism with variable Nissl granules. The granular cells (G) are loose and less packing than the control group. (Cresyl violet X1000)

Figure 11. A photomicrograph of a paramedian section in the vermis of the cerebellum of a rat from group II showing mild pleomorphic destruction (arrow) of myelinated fibers in white matter (W). (Luxol fast blue X40)
Figure 12. A photomicrograph of a paramedian section in the vermis of the cerebellum of a rat from group II showing mild increase in the astrocytes (arrows) and their branches (arrow heads) in comparison with the control group. (Immunohistochemical stain for GFAP X1000)

Figure 13. An electronmicrograph in the vermis of the cerebellum of a rat from group II showing a Purkinje cell with large irregular deformed nucleus (N), increase in mitochondria (M), proliferation of rough endoplasmic reticulum (ER) and increase in free ribosomes (R). Note decrease in Golgi apparatus (G). (Uranyl acetate and lead citrate X5000)

Figure 14. An electronmicrograph in the vermis of the cerebellum of a rat from group II showing granular cells with normal central large oval nucleus (N) and decrease in mitochondria (M) and free ribosomes (R). These cells are surrounded by normal nerve fibers (NF). Microglial cell engulfing electron-dense material (arrow) surrounded by electron-lucent mass (arrow head). (Uranyl acetate and lead citrate X5000)
Figure 15. A photomicrograph of a paramedian section in the vermis of the cerebellum of a rat from group III showing marked oedema and vacuolations (O) in the molecular (M), Purkinje (P) and granular (G) layers. There is marked degeneration and distortion in the Purkinje cells which are smaller in size with pyknotic nuclei. The granular cells are less dense and less packing. The capillaries (C) in the molecular layer are dilated, distorted with destruction of endothelial cells which are found inside the lumen. (Hx & E X400)

Figure 16. A photomicrograph of a paramedian section of the cerebellum of a rat from group III showing marked oedema and vacuolations (O) in the molecular (M), Purkinje (P) and granular (G) layers. Purkinje cells are arranged in many layers and intermingling with granular cells. Some Purkinje cells are degenerated, distorted with pyknotic nuclei (arrow heads). Loss of nuclear cytoplasmic differentiation (short arrows) in many Purkinje cells is noticed. There are decrease in density and packing of granular cells. There is increase in the vascularity with dilatation of the capillaries (long arrows) in the molecular layer. (Hx & E X400)

Figure Kumar, T.A. (2008). A photomicrograph of a paramedian section in the vermis of the cerebellum of a rat from group III showing degeneration and distortion of a Purkinje cells (P) with increase of the intensity of staining for Nissl granules. The granular cells (G) reveal decreased intensity of staining for Nissl granules. (Cresyl violet X 1000)
Figure 18. A photomicrograph of a paramedian section in the vermis of the cerebellum of a rat from group III showing marked pleomorphic destruction (arrows) of the myelinated fibers in white matter (W). (Luxol fast blue X40)

Figure 19. A photomicrograph of a paramedian section in the vermis of the cerebellum of a rat from group III showing astrocytic proliferation (arrows) with increased intensity of immunostaining. In addition, marked increase in thickness and branching of the processes (arrow heads) of astrocytes are noticed. (Immunohistochemical stain for GFAP X1000)

Figure 20. An electronmicrograph in the vermis of the cerebellum of a rat from group III showing a Purkinje cell with deformity and irregularity of the nucleus (N). There are prominent increase in mitochondria (M) and proliferation of rough endoplasmic reticulum (ER). Excess primary (L₁) and secondary (L₂) lysosomes appear. (Uranyl acetate and lead citrate X5000)
Figure 21. An electronmicrograph in the vermis of the cerebellum of a rat from group III showing a granular cell (G) with shrunken deformed irregular nucleus (N) and increased electron density of the cytoplasm. Oedema in the astrocytic processes (arrows) and distortion in the contour of nerve fibers (F) surrounding the granular cell are noticed. A macrophage cell (MC) which contains electron-dense material (arrow heads) is noticed. (Uranyl acetate and lead citrate X5000)