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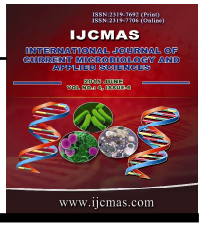

Original Research Article

Effect of Bisphenol-A on the Post-Natal Development and Structure of Rat Cerebellum

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Keywords

Bisphenol A (BPA), Post-natal development and Structure of rat cerebellum

A B S T R A C T

Bisphenol A (BPA) is an estrogenic high production chemical used extensively in plastic industry. The general population is inevitably exposed to BPA because it leaches from containers to food. We aimed to study the toxic effect of BPA on the postnatal developmental structure of cerebellum in adult male albino rat. 40 pregnant rats were used in this study. Four rat groups were given BPA orally at 0 (control), 100, 200, or 400 µg/kg body weight, respectively. BPA were given from gestational day 6 through postnatal day 30. Pups were sacrificed at 1, 3, 6, and 9 wk of age. Cerebellums were dissected, fixed and examined by light and electron microscopes. The body weight and brain weight in BPA treated rats were significantly reduced in comparison to the control groups. In postnatal day 7 in BPA treated rats; BPA increased the thickness of the external granular layer with wide spaces in the deep cells. The molecular layer is thin and immature. The internal granular layer showed a notable degree of immaturity and a much lower number of cells compared to the control group. The cells are scattered unorganized with wide spaces. The granule cells were compact and fused together forming large degenerated necrotic areas. By 3 weeks old rats, most of the Purkinje neurons were not organized. Purkinje cells lacked the principal dendrite. The degenerated areas increased in all layers. The internal granular cell layer thickness was low as compared to the controls. By 6 weeks old rats, cavitations and necrotic areas appeared in all cerebellar layers. Purkinje cells were small in size with abnormal shape and loss the prominent dendrite. The pathological changes were dose dependant. The ultra structure observation of cerebellum in treated animals revealed several damaged and elongated and enlarged mitochondria with destructed cristae. Intracellular calcium deposition was noticed. Purkinje cells in the treated groups were characterized by large and irregular outline nuclei and irregular distribution with abnormal morphology of some organelles. Cerebellar cells with apoptotic changes were visible in pups of dams treated with BPA. BPA has been observed to delay the cytogenesis and morphogenesis of the cerebellum with marked pathological changes.

tanks, supply pipes and components of food

Introduction

Bisphenol-A (BPA) was invented in the 20th century (Patisaul *et al.*, 2006). BPA is an estrogenic high production chemical used extensively in plastic industry and commerce to manufacture baby bottles, water storage packaging as in the lining of food cans (Vandenberg *et al.*, 2009). The general population is inevitably exposed to BPA because it leaches from containers to food.

Exposure to heat increased the rate of BPA migration. BPA is found in various human fluids including fetal serum, maternal serum and full-term amniotic fluid indicating the ability of BPA to pass through the placenta (Yamada *et al.,* 2002; Ikezuki *et al.,* 2002).

The intrauterine foetal life environment is critical for the normal development. The rat cerebellum during the first period after birth is considered to be equivalent to the third trimester of human pregnancy (Hamre and West, 1993; Thomas *et al.,* 1998). Chemicals effects and changes in the levels of hormones can lead to changes in brain function and consequently in behavior (Jacobson and Jacobson, 1996; Faroon *et al.,* 2001). Factors that affect the normal development of the cerebellum can cause pathological effects, depending on the developmental stage (Laure-Kamionowska and Maślińska, 2009).

The cerebellar Purkinje cell is one of the largest neurons and very sensitive to some toxicants like ethanol (Fonnum and Lock, 2000). Maternal administration of BPA causes developmental and reproductive toxicity and behavior abnormality of experimental animal's offspring (vom Saal *et al.,* 1998). BPA interferes with differentiation of ectodermal tissues, including neural tissues, in cynomolgus monkeys (Yamamoto *et al.,* 2007). BPA also induces apoptosis in central neurons of tadpoles resulting in head, vertebral, and abdominal developmental defects (Oka *et al.,* 2003).

This study was designed to assess the effect of BPA on the postnatal developmental structure of cerebellum in adult male albino rat.

Material and Methods

40 pregnant 9-wk-old rats purchased from animal house at gestation day 3. They were individually housed under a 12/12 h light/dark cycle, with free access to feed and tap water. Room temperature and humidity were maintained at 23 ±1° C and 55 ± 5%, respectively. The handling of animals followed the rules for experimental research ethics approved by Research Ethics Committee at King Khaled University (REC≠2014-06-03). Four rat groups, of 10 pregnant rats each, were given standard BPA at 0 (control), 100, 200, or 400 µg/kg body weight (BW), respectively. BPA were dissolved in corn oil (10 ml/kg BW). BPA were administered to rats by oral gavages from gestational day 6 through postnatal day 30. Pups were sacrificed at 1, 3, 6, and 9 weeks of age. Forty neonatal rats from all groups were sacrificed every time at age of one, three, six and nine weeks.

The weight of each animal was recorded. The rats were anesthetized with diethyl ether and sacrificing, the brain of each rat was dissected and removed and its weight was recorded. Cerebellums were dissected then fixed for histopathological examinations.

Chemicals

All chemicals were obtained from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA).

Light microscopy

All specimens for light microscope examination were cut into small pieces and fixed in a solution of 10% formaldehyde and processed to get paraffin sections of 5 µm thickness. Sections were stained with Haematoxylin and Eosin (H&E) (Bancroft and Stevens, 1996). Slides were mounted using entellan and covered with cover slips prior to viewing and photography by (Nikon Eclipse E-200) light microscope.

Transmission electron microscopy

The cerebellum specimens were cut into small pieces of 1mm3 sizes and fixed in 2.5% glutaraldehyde for 24 hours. Specimens were washed in 0.1 M phosphate buffer at 4°C, then post fixed in 1% osmium tetroxide at room temperature. Specimens were dehydrated in ascending grades of ethyl alcohol, and then embedded in Epon resin. Semi thin sections (1μm) were stained with toluidine blue in borax and examined with light microscope. Ultrathin sections (50 nm) were cut, mounted on copper grids and stained with uranyl acetate and lead citrate. Specimens were examined and photographed with Jeol 1200 EX transmission electron microscope in College of Medicine, King Khaled University.

Data analysis

Data were presented as mean and standard error of means. The degree of significance was set at $P < 0.05$. The changes in body weights and brain weights and structural changes were analyzed by two-way analysis of variance. All data analyses were carried out using the SPSS 15.

Results and Discussion

The results revealed more prominent signs of toxicity in rats treated with BPA, where most treated rats became less active and showed general weakness. Also, they had lost their appetite and showed loss in their body sizes. BPA treated pups grew at a slower rate.

The body and brain weights were recorded to be significantly low in the BPA treated animals as compared to their respective controls.

Table.1 Effect of BPA on average body weight and brain weight (gm) during postnatal development of rat cerebellum.

Group	Body weight/ 1week	Body weight/ 3 weeks	Body weight/ 6 weeks	Body weight/ 9 weeks	Brain weight/ 1 week	Brain weight/ 3 weeks	Brain weight/ 6 weeks	Brain weight/ 9 weeks
I	11.45 ±0.16	46.71 ±0.64	120.35 ±1.89	195.97 ±2.71	1.04 ±0.05	1.57 ±0.07	1.86 ±0.08	2.01 ±0.08
II	10.31 ±0.14	42.76 ±0.59	112.75 ±1.77	184.09 ±2.57	0.96 ±0.04	1.48 ±0.06	1.79 ±0.07	1.92 ±0.08
III	9.28 ±0.13	38.86 ±0.54	105.73 ±1.66	177.64 ±1.48	0.90 ±0.04	1.40 ±0.05	1.70 ±0.07	1.86 ±0.08
IV	8.45 ±0.11	35.11 ±0.53	97.99 ±1.58	169.98 ±1.38	0.87 ±0.03	1.35 ±0.04	1.65 ±0.05	1.80 ±0.07

Light microscopic result

The cerebellar cortex of neonatal rat one week old is seen to be consisted of four layers: the outermost external granular layer which covering the surface of the developing cerebellum, molecular layer, Purkinje cell layer and an inner granular layer (Fig. 1). The external granular layer is formed of four to five rows of cells. The molecular layer is thin layer and immature with few number of basket cells.

The Purkinje cells in the third layer are arranged at the junction between the

molecular and the granular layers. They are nearly mature cells. The internal granular layer is thick and contains small neurons called the granule cells, which have large, rounded nuclei and scanty cytoplasm (Fig. 1).

Effect of BPA low dose on the neonatal rat one week old revealed the presence of the external granular layer nearly the same size of the control group. Many cavitations were present deep to the external granular layer as a sign of necrosis and degeneration. Purkinje cells were immature. Cavitations and necrosis were also present in the internal granular layer (Fig. 2).

In group III, Effect of BPA was most obvious in the external granular layer. The external granular layer was well recognized, thicker than the control group and formed of five to six rows of cells. The deep cells are separated from each others with wide spaces and some cells were seen in the molecular layer. The molecular layer is thin and immature with few numbers of cells. The molecular layer had marked degenerated areas. Purkinje cells showed immature and abnormal shape (Fig. 3). They revealed degenerative changes with loss of their normal pyriform shaped appearance. The internal granular layer showed a notable degree of immaturity and a much lower number of cells compared to the control group. The cells are scattered unorganized with wide spaces. The granular cells were compact and fused together forming large degenerated necrotic areas, and revealing signs of injury and they had lost their normal organization. Many cells were mostly pyknotic (Fig. 3).

In high dose of BPA, group IV; the external granular layer was also well recognizable and thicker compared to the control group. The deep cells are separated from each others with wide intercellular spaces and some cells were seen in the molecular layer. In comparison with the controls, many cells of this layer had enlarged nuclei and some underwent shrinkage necrosis and karyorehexis. The area between the external granular layer and the molecular layer was irregular and not marked. The molecular layer is nearly has the same pathological changes as group III. Purkinje cells showed marked immature and abnormal shape (Fig. 4). The Purkinje cells layer revealed marked degenerative changes. The internal granular layer showed a higher degree of immaturity and a much lower number of cells compared to the control group or group II or even group III. The cells are scattered unorganized with more wide spaces. Pyknotic cells were present. The granule cells were degenerated with large and wide necrotic areas, and revealing severe signs of apoptosis, damage and destruction of cerebellar tissue (Fig. 4). Thickness of this layer was low in the BPA-treated animals as compared to the controls. The marked pathological changes were marked in one week old rats treated with BPA 400 ug/ kg BW. In all treated groups, the pathological changes were dose dependant.

The tissue lesions were more advanced. Pathological changes in cerebellum of neonatal rats treated with BPA demonstrated the features of nervous tissue damage expressed by marked cellular damage. Focal degeneration of the tissue has also been noticed. The observed changes suggested necrosis or apoptosis of the cells.

In control group preparations, 3 weeks old rats, the external granular layer markedly reduced in size to be a single or double rows layer or disappear completely in certain area. Molecular layer increased in thickness and consisted of basket and stellate cells. Purkinje cells achieved the typical flask shape with streaming up of the apical

cytoplasm. The cells are arranged in a single row. The internal granular layer was thick with many prominent granular cells (Fig. 5). The granular cell layer thickness increased in three weeks old age rats.

The external granular layer, in treated animals group II, reduced to a single raw layer in the pups at three weeks old age. In the same aged treated pups, most of the Purkinje neurons were not will organized in the process of monolayer formation. Purkinje cells lacked the principal dendrite or even the apical cone. A narrower Purkinje cell layer was revealed in three weeks BPA-treated animals. An increased packing density of the Purkinje neurons was also prominent (Fig. 6).

In group III, the cavitations and degenerated areas increased in all layers. Some Purkinje cells were abnormal in shape. The internal granular layer has some pyknotic nuclei (Fig. 7). While in group IV, a low molecular layer thickness was reduced and the cavitations increased. Purkinje cells reduced in number with abnormal shape, loss of major dendrite and some cells revealed degeneration (Fig. 8). The internal granular cell layer thickness was low in three weeks old age BPA-treated group's preparations as compared to the controls.

Subsequently, by 6 weeks old rats, the external granular layer of cerebellum completely disappeared in all groups. However, in 6 weeks old rats preparations there was increase in thickness in the molecular and internal granular layers in controlled animals. Dendrite of Purkinje cells was prominent and noticed with dispersion and alignment in monolayer (Fig. 9). In BPA treated groups, cavitations and necrotic areas appeared in all cerebellar layers. Some Purkinje cells were small in size with abnormal shape and loss of the prominent dendrite. Also the arrangement is impaired and disorganized (Fig. 10). Thickness of the internal granular layer also remained low in the BPA -treated animals as compared to the controls.

In 9 weeks old rats, control group, the cerebellar cortex was formed of three full mature layers: the outermost molecular layer, an inner granular layer and a central Purkinje cell layer (Fig. 11). The molecular layer contains glial cells, few neurons, those of stellate and few scattered nuclei of basket cells (Fig. 11). The Purkinje cells in the second layer have large flask-shaped appearance, and they are arranged in a single row between the molecular and the granular layers. Each cell has a conspicuous cell body, centrally vesicular nuclei and an extensive fan -like dendrite tree (Fig. 11). The granular layer contains numerous granule cells, which have large, rounded nuclei and scanty cytoplasm (Figs.11). While in BPA treated groups, the same pathological changes of six weeks old treated rats were noticed (Figs. 12 & 13). The total cerebellar cortex thickness also remained low in the BPA -treated animals as compared to the controls during the entire period of study.

Ultra structure study

Rats from control group showed normal histological structure of the cerebellum for the developmental age of the examined animals. Purkinje cells in the control one week old rat showed a nearly mature morphology. All their cytoplasm was filled with cellular organelles, especially the mitochondria, free ribosomes, rough and smooth endoplasmic reticulum (Fig. 14). Granular cell layer of three weeks old rat in control group revealed closed granular cells with large oval nuclei and cytoplasm full of organelles. Some myelinated and unmyelinated nerve fibres were present between granular cells (Fig. 15).

The ultra structure observation of cerebellum in treated animals with BPA revealed pathological lesions in the tissue. One week old rat treated with BPA revealed damaged Purkinje cells characterized by large nuclei often irregular in outline and irregular distribution of organelles in the cytoplasm. Elongated mitochondria with destructed cristae and some calcium deposition in the cytoplasm, mitochondria and endoplasmic reticulum were also noticed. The nerve fibers have no myelin sheets and most of synapses were immature (Fig. 16). The cytoplasm was filled with numerous free ribosomes. Free ribosomes were noticed accumulating in certain area of cytoplasm. Numerous dilated channels of rough and smooth endoplasmic reticulum were seen also in the treated groups. The morphology and organization of mitochondria is impaired (Fig. 17). Nucleus has irregular outline and shrunken. In all experimental groups treated with BPA the ultra structural changes described above, were accompanied by the degenerative damage of Purkinje cells that varied in intensity and dose dependant.

The molecular layer of one week old treated rat even with low dose of BPA revealed immature nerve fibers; most of them are unmyelinated and immature synapses. Granular cell from the external granular layer was characterized by irregular shaped dark nucleus with dark nucleolus. Abnormal shaped mitochondria, dilatation of the smooth endoplasmic reticulum with free ribosomes accumulating in certain area of the cytoplasm and calcium deposition were noticed (Fig. 18). All these changes indicate pathological damage of the molecular and granular layers due to BPA treatment.

Pathological features of apoptosis were found in the external granular layer of cerebellum treated with high dose of BPA. They showed the condensed chromatin under the nuclear membrane and shrunken of some nuclei and dense cytoplasm (Fig. 19). Dark neurons and cavitations were noticed. Dying cells with morphological features of apoptosis were found in the external granular layer and Purkinje cells (Fig. 20). The majority of cells with apoptotic changes were visible in pups of dams treated with BPA in all layers.

The internal granular layer of BPA treated animals showed shrinkage of some nuclei and abnormal mitochondria. There were also wide extracellular space (Fig. 21) indicating apoptotic and degenerative cellular changes. Treatment with BPA has been observed to delay the cytogenesis and morphogenesis of the cerebellum.

Fig.1 A photomicrograph of cerebellar tissue of a rat from the control group one week old showing the external granular layer(E), some basket cells (b) appeared in the molecular layer (M), Purkinje cell layer (P) and internal granular layer (G). (H&E X 400)

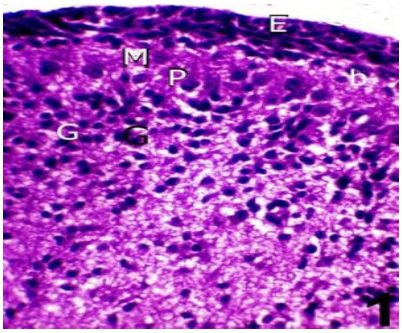


Fig.2 A photomicrograph of cerebellar tissue of a rat from the group (II) one week old shows the external granular layer (E), molecular layer (M), Purkinje cell layer (P) and internal granular layer (G). Notice the presence of much area of necrosis and degeneration represented as cavitations (C). (H&E X 200)

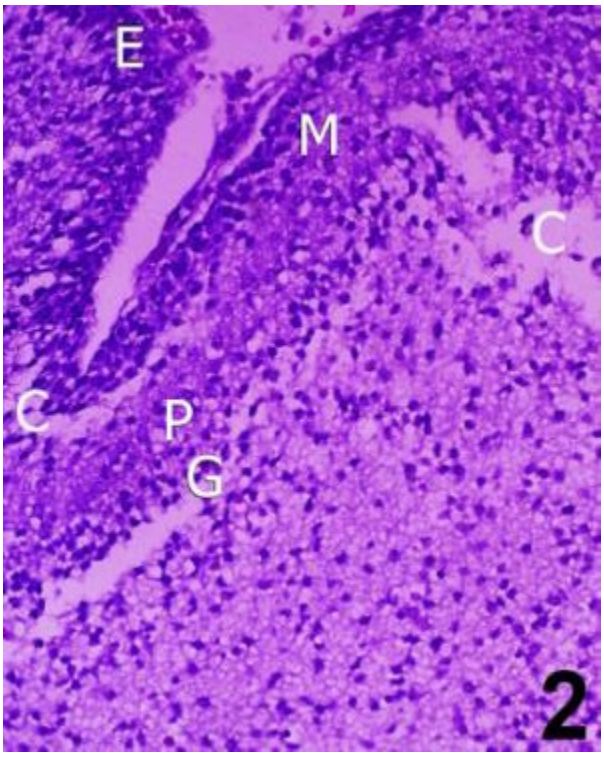


Fig.3 A photomicrograph of cerebellar tissue of a rat from the group (III) one week old showing the external granular layer(E) with wide intercellular spaces (arrow) and degenerated area (C), thin molecular layer (M), abnormal and disorganized Purkinje cell layer (P) and internal granular layer (G) with some pyknotic cells (*) and marked degenerated area (C). (H&E X 400)

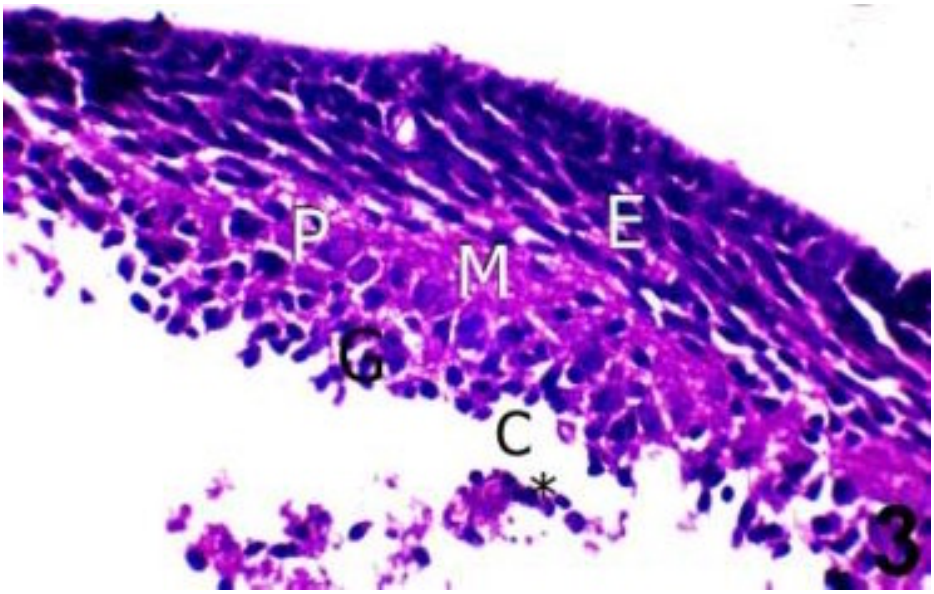


Fig.4 A photomicrograph of cerebellar tissue of a rat from the group (IV) one week old showing the external granular layer (E) with increased thickness and wide intercellular spaces (arrow) and degenerated area (C), thin molecular layer (M), abnormal and disorganized Purkinje cell layer (P) and internal granular layer (G) with some pyknotic cells (*) and marked degenerated area (C). (H&E X 400)

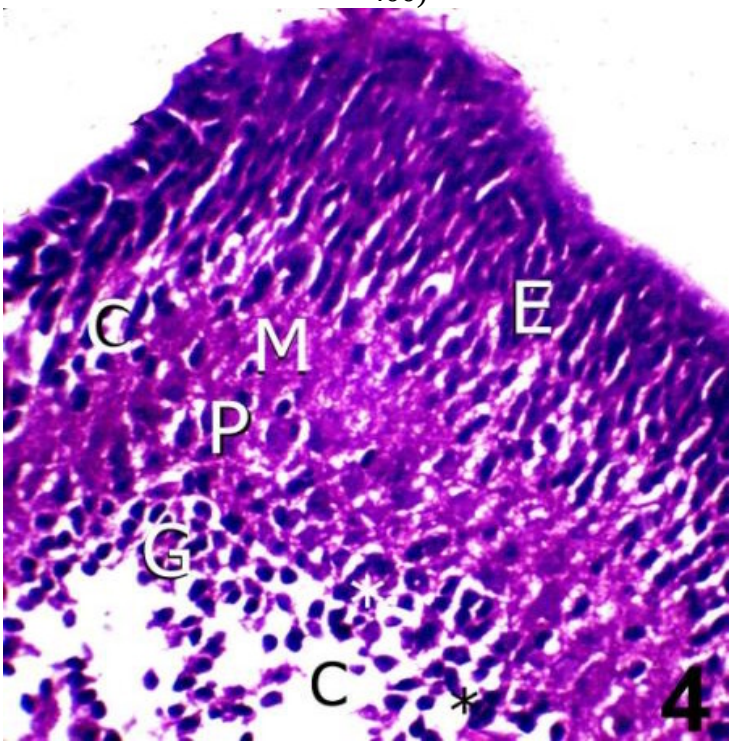


Fig.5 A photomicrograph of cerebellar tissue of a rat from the control group three weeks old showing the external granular layer(E) very thin and disappear in certain area, molecular layer (M) with stellate (s) and basket cells (b), Purkinje cell arranged in single layer (P) and internal granular layer (G). (H&E X 400)

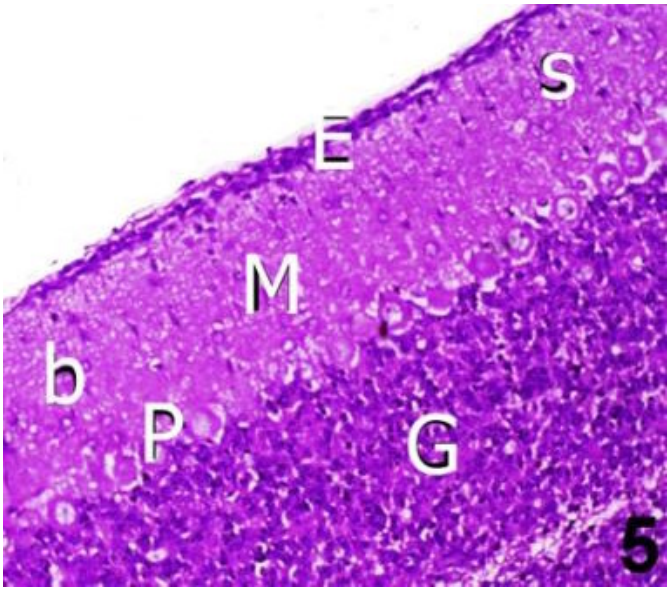


Fig.6 A photomicrograph of cerebellar tissue of a rat from group (II) three weeks old showing the external granular layer (E) very thin and disappear in certain area, molecular layer (M) with stellate (s) and basket cells (b), Purkinje cell arranged in single layer (P) with abnormal morphology and degenerated area (C) and internal granular layer (G) with pyknotic cells (*). (H&E X 400)

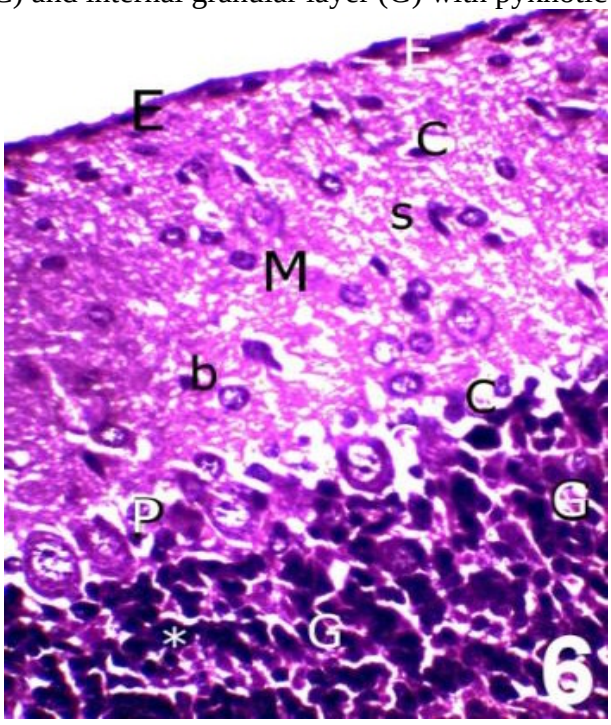


Fig.7 A photomicrograph of cerebellar tissue of a rat from group (III) three weeks old showing the external granular layer(E) very thin layer, molecular layer (M) with stellate (s) and basket cells (b), Purkinje cell layer (P) with abnormal morphology and degenerated area (C) and internal granular layer (G) with pyknotic cells (*). (H&E X 400)

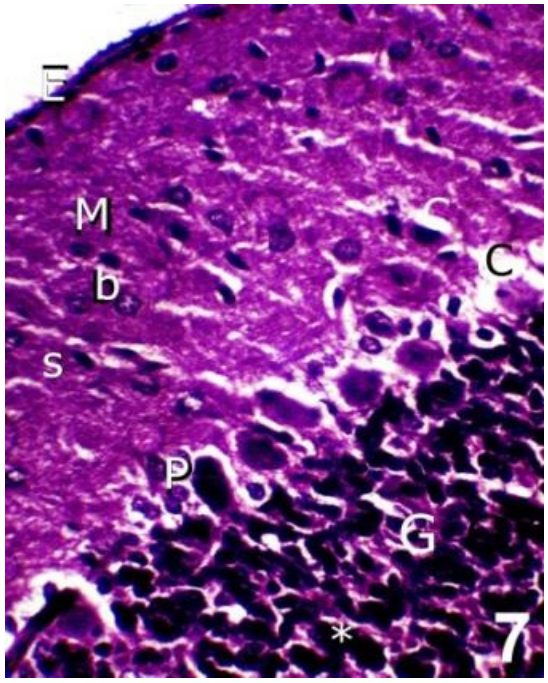


Fig.8 A photomicrograph of cerebellar tissue of a rat from group (IV) three weeks old showing the external granular layer (E) very thin layer, molecular layer (M) thin compared to the control group with stellate (s) and basket cells (b), Purkinje cell layer (P) with abnormal morphology and marked degenerated area (C) and internal granular layer (G) with pyknotic cells (*) and degenerated area (C). (H&E X 400)

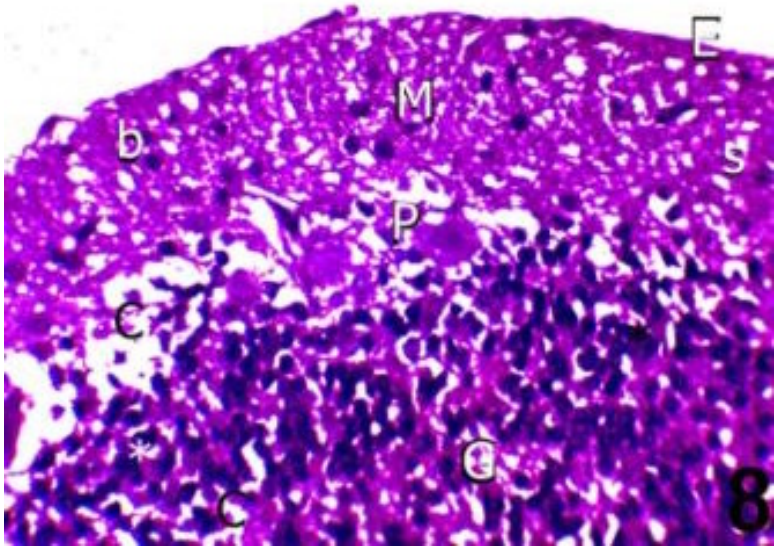


Fig.9 A photomicrograph of cerebellar tissue of a rat from the control group six weeks old showing, molecular layer (M) with stellate (s) and basket cells (b), Purkinje cell arranged in single layer (P) with long dendrite (D) and internal granular layer (G). (H&E X 200)

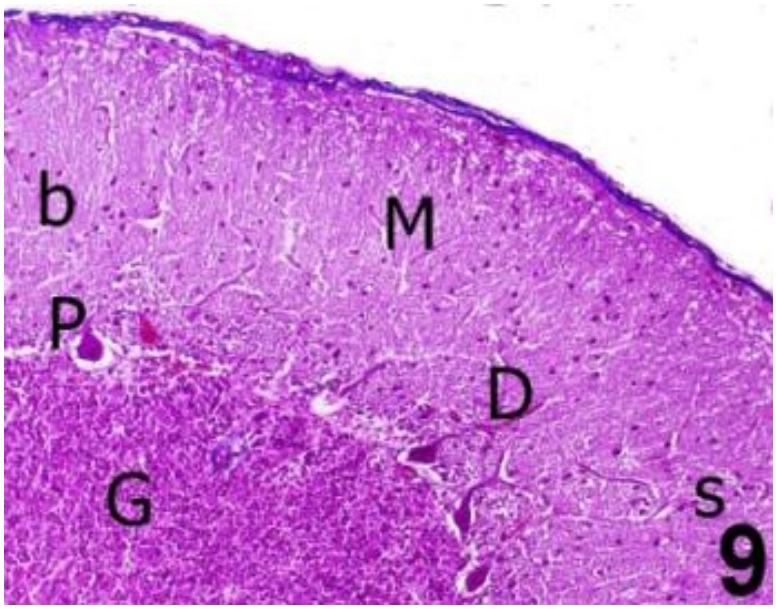


Fig.10 A photomicrograph of cerebellar tissue of a rat from group (III) six weeks old showing, molecular layer (M) with marked area of degeneration (C), Purkinje cell disturbing layer (P) with abnormal morphology and internal granular layer (G) with pyknotic cells (*) and degenerated areas (C). (H&E X 400)

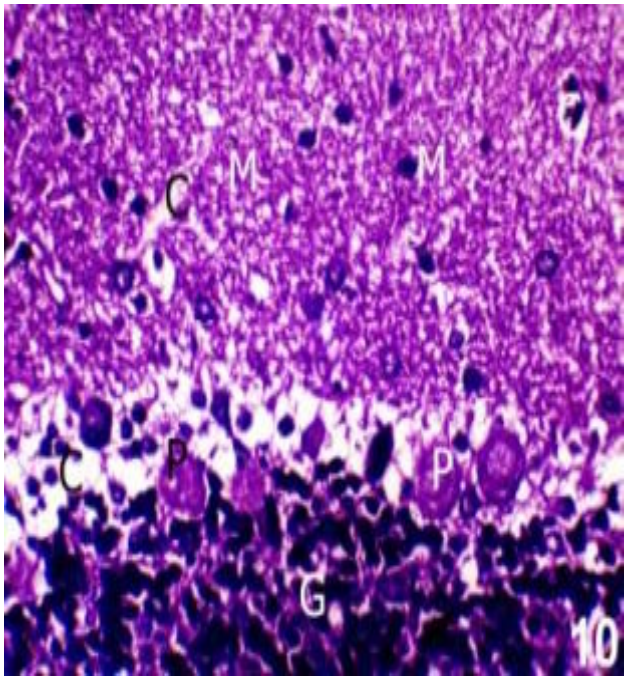


Fig.11 A photomicrograph of cerebellar tissue of a rat from the control group nine weeks old showing, molecular layer (M) with stellate (s) and basket cells (b), Purkinje cell arranged in single

layer (P) with long dendrites (D) and internal granular layer (G). (H&E X 200)

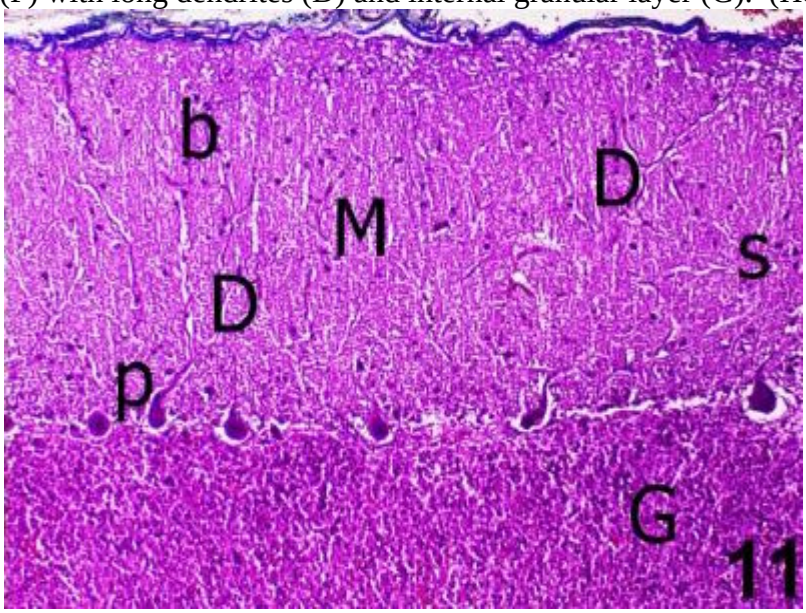


Fig.12 A photomicrograph of cerebellar tissue of a rat from group (III) nine weeks old showing, molecular layer (M) with area of degeneration (C), Purkinje cell disturbing layer (P) with abnormal morphology. Some cells are small and shrunken with loss of major dendrites. Notice the internal granular layer (G) with pyknotic cells (*) and degenerated areas (C). (H&E X 400)

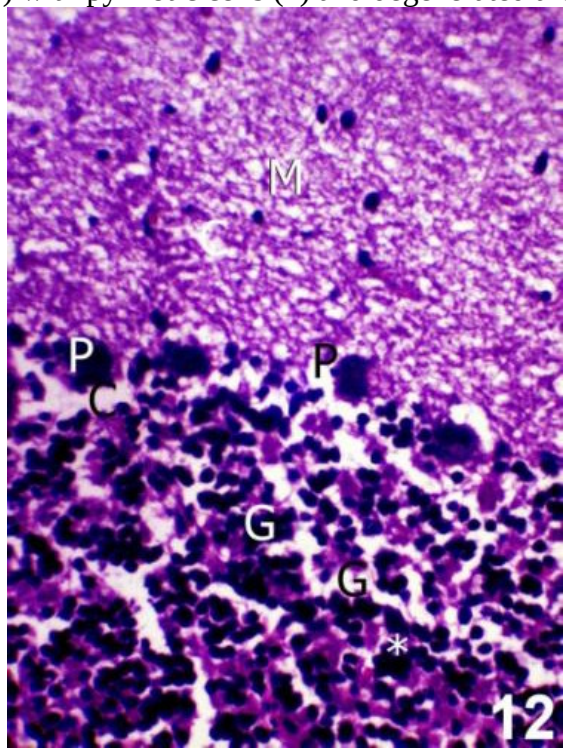


Fig.13 A photomicrograph of cerebellar tissue of a rat from group (IV) nine weeks old showing, molecular layer (M) and area of degeneration (C), Purkinje cell disturbing layer (P) with

abnormal morphology and internal granular layer (G) with pyknotic cells (*) and degenerated areas (C). (H&E X 400)

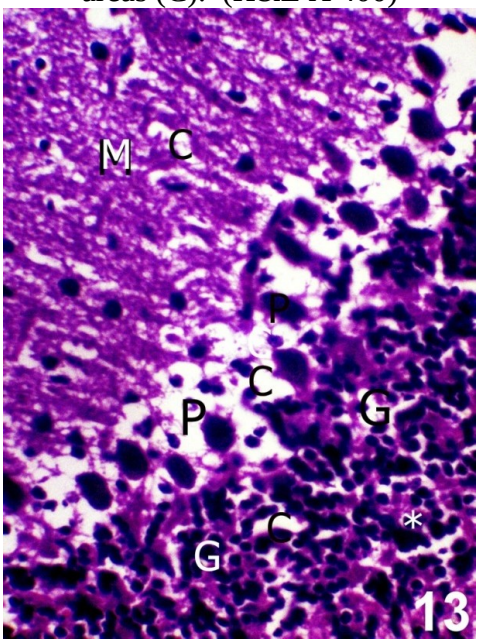


Fig.14 Electron micrograph of rat's cerebellar sections of control group one week old. Normal nerve fibers (f) and Purkinje cell with large nucleus (N), mitochondria (m), rough (R*) and smooth (R) endoplasmic reticulum and free ribosomes (r). (X 10000)

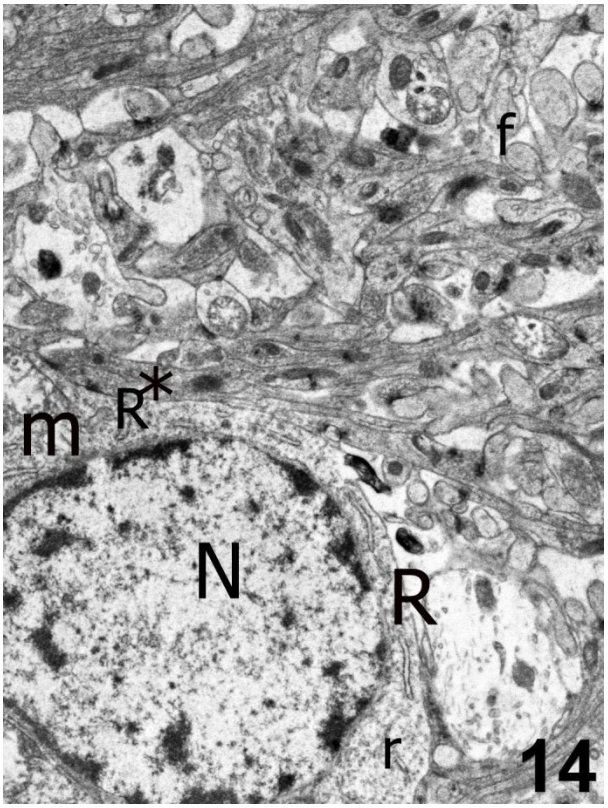


Fig.15 Electron micrograph of rat's cerebellar sections of control group three weeks old shows normal myelinated nerve fibers (f) and many granular cells with large and oval nucleus (N). (X 10000)

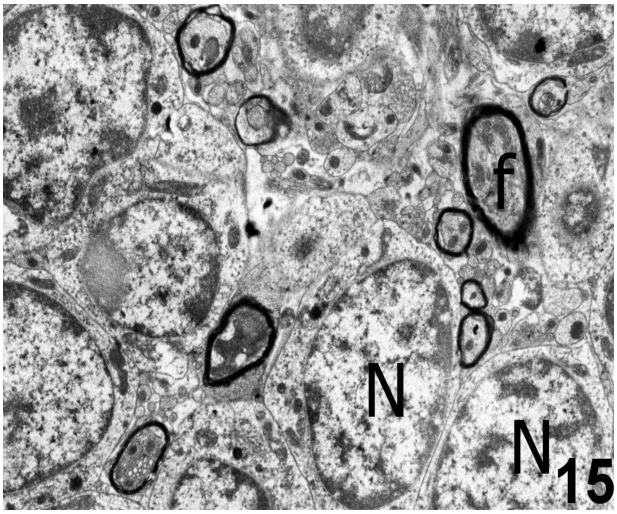


Fig.16 Electron micrograph of rat's cerebellar sections of group (III) 7 days old shows nerve fibers and Purkinje cell with aggregated and dilated smooth endoplasmic reticulum (r), enlarged mitochondria (m), and free ribosomes (R). Numerous vesicles containing calcium deposits (*) are present in damaged cell, mitochondria and inside the nerve fibers. (X 10000).

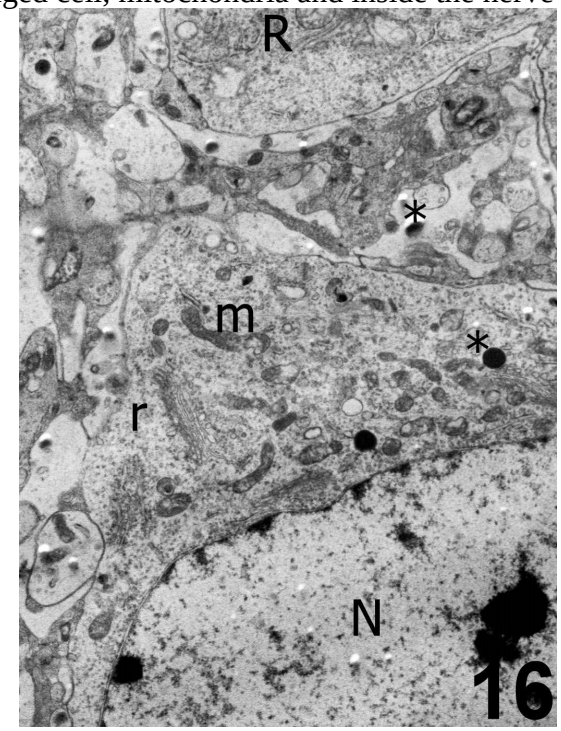


Fig.17 Electron micrograph of rat's cerebellar sections of group (III) three weeks old, revealing immature nerve fibers and Purkinje cell with irregular outline and shrunken nucleus (N),

aggregated and dilated smooth endoplasmic reticulum (E), marked enlarged mitochondria (m) with destructed cristae and free accumulating ribosomes (r).(X 10000)

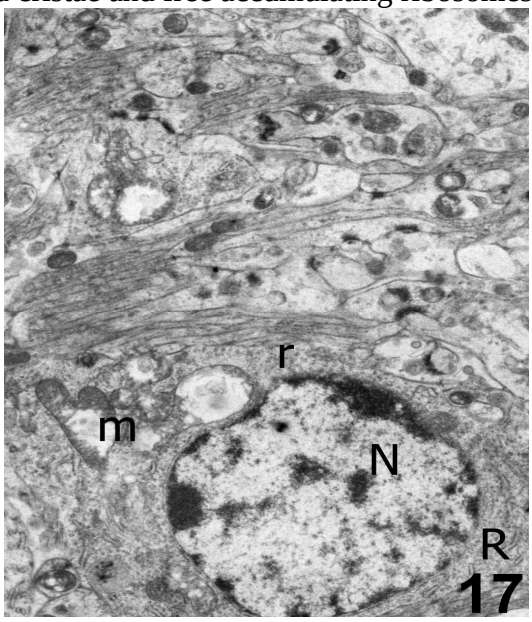


Fig.18 Electron micrograph of rat's cerebellar sections of group (II) one week old, revealing immature nerve fibers and granular cell from the external granular layer with irregular shaped dark nucleus (N) with dark nucleolus, abnormal mitochondria (m), free ribosomes (r) and calcium deposit (*). Notice the dilatation of the smooth endoplasmic reticulum (R). (X 10000)

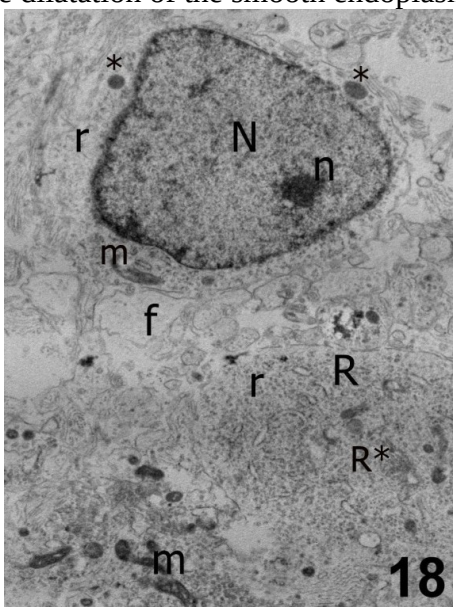


Fig.19 Electron micrograph of rat's cerebellar sections of group (IV) Three weeks old, revealing dark nerve fibers (f) and granular cell from the external granular layer with irregular shaped dark nucleus (N), abnormal mitochondria (m), free ribosomes (r) and large cavities (C). (X 10000)

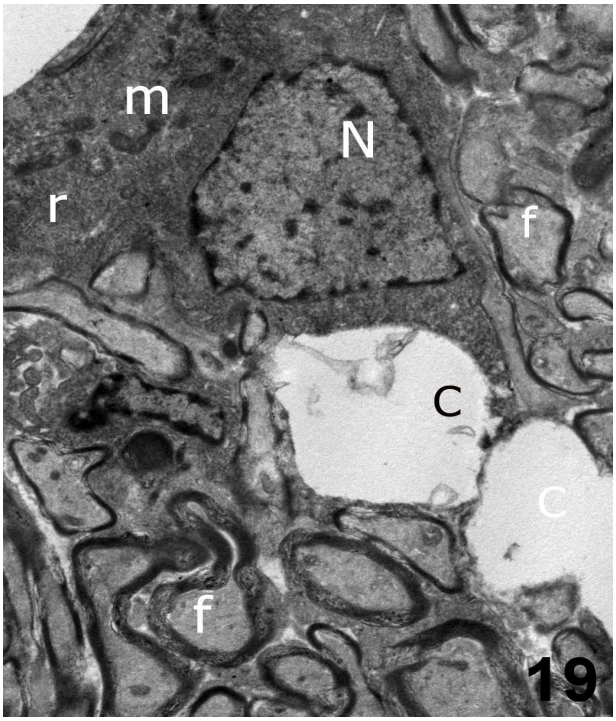


Fig.20 Electron micrograph of rats cerebellar sections of group (IV) Three weeks old, revealing dark nerve fibers (f) and granular cell from the external granular layer with irregular shaped dark nucleus (N), apoptotic Purkinje cell (P) and large cavity (C). (X 5000)

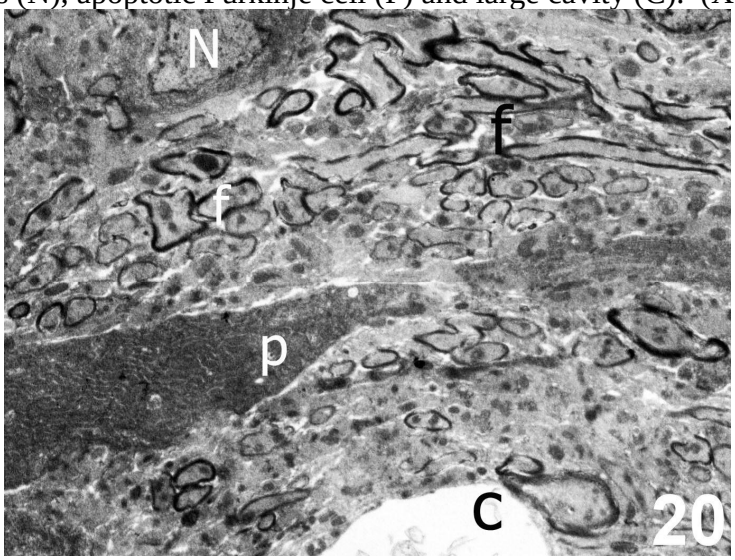
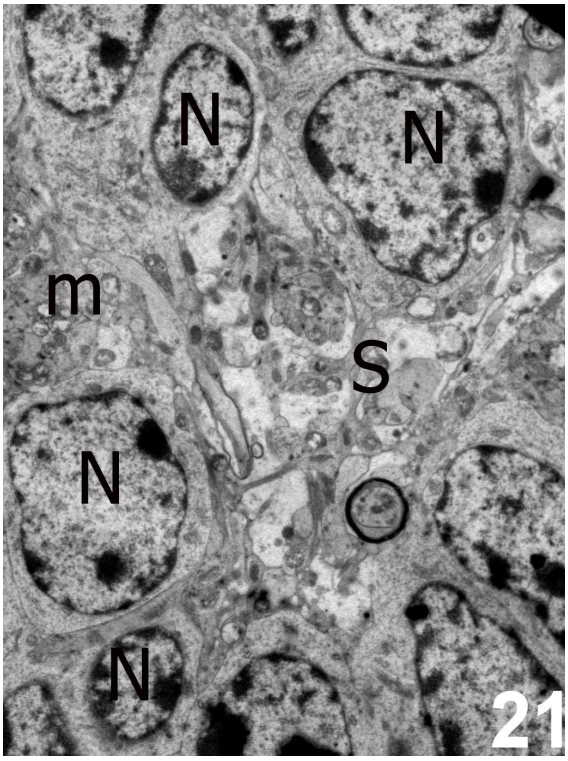


Fig.21 A transmission electron micrograph of rat's cerebellar sections three weeks old of the group IV, showing shrinkage of some nuclei (N) of the internal granular cell layer, abnormal mitochondria (m). Note wide extracellular space (S). (X 5000)



Development of mammalian brain is highly sensitive to toxins, as compared to the adult brain, with permanent pathological damage. Developmental neurotoxicity researches demonstrate the histopathological effects caused by drugs, pesticides and occupational hazards otherwise claimed to be comparatively safe to adult brain. Exposure to arsenic, lead and chloroquine during brain development has been recorded to affect the histogenesis and morphogenesis of cerebellum (Pounds, 1984; Agarwal, 1991). Pathological effects produced by these factors will affect the normal development of the cerebellum, depending on the developmental stage (Laure-Kamionowska and Maślińska, 2009). The cerebellum is highly affected by the environmental factors that Leeds to undifferentiated neural cells and destruction of the neurons (Koestner and Norton, 1991; Inouye, 1995). Congenital brain anomalies may be caused by disturbance of the migrating neurons (Barth, 1987; Hatten, 1990).

The first period after birth of rat cerebellum is considered to be equivalent to the third trimester of human pregnancy (Thomas *et al.*, 1998). Three weeks of age are equivalent to the first and have years of the human postnatal life (Sengupta, 2013). Most of the cerebellum cells and its neurons are formed during the postnatal life (Balazas and Patel, 1973). Granular cell proliferation and migration, formation of the cells of the molecular layer, Purkinje cell full maturation and synaptic formation occurred normally during the first three weeks of postnatal development of rat cerebellum (Epstein and Stanford, 1977).

The present study revealed full maturity of rat cerebellum after nine weeks. The rat cerebellum reaches its total maturity in the third month of life (McKay and Turner, 2005; Jortner, 2006).

Gopinath (Gopinath, 1984) observed that the brain weight was low in the experimental under nutrition. BPA exposure also decreases the body and brain weight dose dependant and may be due to loss of animal's appetite and activities.

The external granular layer at age of one week are formed of four to five layers while in BPA treated groups this layer is thicker and formed of six to seven rows. Thickness of the external granular layer and migration of granular cells have also been reported in treatment with corticosteroids (Cotterrell *et al.,* 1972). The external granular cells beginning after birth until three weeks old age when it starts to disappears (Altman and Anderson, 1972, 1973). Most of cells of the external granular layer disappear at the end of the third week, while the granule cells proliferate and migrate deep to the Purkinje cell layer to form the inner granular layer (Clos *et al.,* 1977). Similar results have been recorded during the present study in the control group. This may be due to slow migration of neurons as evident from the thick layer and mitotic division or may be due to cell proliferation. Enlargement of nuclei may be due to ploidy observed as in glial cells tumour (Fleischmannova *et al.,* 1982).

Such results are in agreement with the previous studies on the cell proliferation seen as a result of under nutrition (Gopinath, 1984; Barnes and Altman, 1973). BPA treated animals, adds further evidence to the fact that BPA delays differentiation of neurons in the cerebellum.

The basket cells are produced mostly at the end of the first week of postnatal age while the stellate cells appeared at the end of the third week. The molecular layer increase in size after first week with the onset of the development of the Purkinje cell dendrites (Altman, 1972). In this research the animals exposed to BPA had low molecular layer thickness as compared to their respective controls. Narrow molecular layer may result due to the reduced dendrites development of the Purkinje cells (Gopinath, 1984).

The cerebellar Purkinje cell is sensitive to some toxicants, like ethanol (Fonnum and Lock, 2000; Lewandowska *et al.,* 2012). Alcohol administration leads to a delayed maturation with decrease in the density of the Purkinje cells (Volk *et al.,* 1981). The present investigation revealed that, BPA leads to delayed maturation of the Purkinje cells with abnormal morphology and pathological degenerative changes. A delay in monolayer arrangement of Purkinje cells in treated animals suggests a general retardation of histogenesis and morphogenesis of the cerebellum. Such adverse effects have also been reported in under nutrition, low protein diet, irradiation and chloroquine toxicity (Agarwal, 1991; Choudhary *et al.,* 1982; Gopinath et al.., 1987).

BPA-treated pups had marked necrotic area in the granular layer, particularly at three weeks old rats. The brain during early development has more neurons, neural branches and synapses. Destruction and loss of some neural elements has important implications in the development of the nervous system (Purves and Lichtman, 1985).

The process of production of excessive neural elements and pruning is an epigenetic strategy for matching the neuronal centers and their targets as a feature of competition (Purves *et al.,* 1996). Pyknotic cells appeared is likely that these cells underwent necrosis because of unrepaired damage at the genome level. The presence of phagocytosis suggests the disposal of cell debris. Some immature astrocytes are activated to phagocytic cells (Korínková *et*

al., 1977).
In BPA-treated animals the formation of the cells of the inner granular layer is delayed and retarded with many pathological changes.

The necrotizing effect of BPA was present in all layers of the cerebellum. The necrosis may be due to the effect of BPA on the DNA or the cellular metabolic processes. BPA has been observed to generally delay the process of cytogenesis and morphogenesis of various types of neurons. BPA may interfere with the initial development of the cerebellum. The toxic effect of BPA during pregnancy causes different pathological changes in the post natal development pubs, including degenerative changes in the cerebellum observed in this study. Thus the present study opens up several questions on the safety of BPA to the new-born babies and adult.

Ultra structural changes

Our electron microscopic study of cerebellar cortex revealed that the major alterations concerned disorders of morphology of Purkinje cells and their degeneration, as well as the degeneration of granular layer cells in all experimental groups.

Purkinje cells in BPA treated groups were filled with cellular organelles, but the arrangement and distribution of ribosomes and endoplasmic reticulum were changed. Ribosomes were arranged and localized in certain areas. The delayed maturation of Purkinje cells may be associated with a decreased ribosomal protein biosynthesis. Accumulation of free ribosomes in cytoplasm was observed in the maturing Purkinje cells. BPA treatment induces alterations in the localization of ribosomes, which may affect the capacity of protein synthesis in neural cells in the neonatal rat brain (Rawat, 1975).

Rough and smooth endoplasmic reticulum were enlarged and dilated. As evidenced endoplasmic reticulum plays a major role in detoxification. Delayed cytoplasmic maturation of Purkinje cells mainly involves the endoplasmic reticulum (Volk *et al.,* 1981).

Ultra structural changes in endoplasmic reticulum of Purkinje cells may influence BPA metabolism. BPA can also be oxidative stress that generates hydrogen peroxide.[42] The production of free radicals derived from hydrogen peroxide leads to oxidative stress and cell death. Free radicals are highly reactive to all the molecular targets by modifying their chemical structure and generating oxidation-derived products.

The degenerating Purkinje cells appeared as “dark” ischemic neurons may be responsible for some phase of apoptosis (Ratan *et al.,* 1994). The same picture was noticed in various pathological processes and the action toxins (Sobaniec-Łotowska and Łotowska, 2011; Wierzba-Bobrowicz *et al.,* 2011). The present research showed degenerating shrunken and dark like Purkinje cells in all experimental groups after BPA intoxication in the developing cerebellum. Purkinje cells in experimental encephalopathy induced by chronic application of valproate showed the same pathological features (Sobaniec-Łotowska and Łotowska, 2011). Dark neurons showed features of aponecrosis (Sobaniec-Łotowska and Łotowska, 2011). Dying cells of the granular layer revealed condensation of chromatin along nuclear membrane, dense clumps and shrinkage of nuclei and cytoplasm.

Mitochondria were markedly damaged reflecting their role as a central organelle mediating cellular death (Kroemer *et al.,* 1998). Calcium is one of the major signaling compounds in the human body (Berridge *et al.,* 1998). Calcium deposition was noticed

early after one week of postnatal life in BPA treated animals. Deposition of calcium was noticed in different areas of cytoplasm. Calcium accumulation in the mitochondria may trigger mitochondrial permeability transition characterized by mitochondrial swelling (Zoratti and Szabo, 1995). Swelling of some mitochondria may lead to the release of proapoptotic proteins leading to the activation of caspase-3 and DNA fragmentation (Kroemer *et al.,* 1998). Calcium deposition seems to be important for the presence of pathological lesions in BPA treated animals. Calcium has influences on the beginning and consecutive steps of brain development but may change the signal of life into that of death.

Accumulation of calcium in neuronal and other tissues may represent the pathway for cell death arising from hypoxia because of the disruption of intracellular calcium homeostasis (Stein and Vannucci, 1988).

Pathological conditions from calcium deposition Leeds to increase permeability of the cellular membrane and increase the calcium cytosol level, which may enter the cells, leading to their destruction by activating some enzymes such as lipases, proteases, and endonucleases (Sjesjo and Bengston, 1989). This may lead to apoptosis or necrotic cell death (Pulera *et al.,* 1998).

Electron microscopic examination of cerebellar cortex revealed pathological changes of Purkinje cells and their degeneration, as well as the degeneration of granular layer cells in all BPA treated groups.

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