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Original Article

A study on the effect of cisplatin administration on the developing cerebellum in the albino rat and possible protective role of alpha lipoic acid

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ABSTRACT

The Cerebellum is highly organized structure. The aim of the present work is to study development of the cerebellum and effect of cisplatin on its development. Also, the possible protective role of alpha lipoic acid has been investigated. A total number of 40 pregnant females Albino rats were used. They divided into 4 groups: control group, cisplatin received group, alpha lipoic acid received group and both alpha lipoic acid and cisplatin received group. The rats received the treatment throughout the period of pregnancy and during the period of lactation. The offspring of rats at newly born, 10 days and 20 days old rats were sacrificed. The present study showed that cisplatin causes degeneration of the neurons in the purkinje layer and granular layer in the cerebellar cortex and in deep cerebellar nuclei. Also, delayed development of the cerebellum could be observed. Morphometric results revealed statistically significant decrease in the number of purkinje and granule cells in cisplatin received group as compared with control group. Most of degenerative changes are improved by alpha lipoic acid.

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Key Words: Alpha lipoic acid, cerebellum, cisplatin, immunohistochemistry, postnatal development, ultrastructural study.

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INTRODUCTION

The Cerebellum is a highly organized structure and its postnatal development was characterized by cell proliferation, migration and differentiation. The cerebellum is involved in the control of movements, particularly those linked to the voluntary nervous system.

It coordinates the different muscle groups so that the muscle exerts movements fluently and precisely. The Purkinje cells are the sole output of the cerebellar cortex[1]. Significantly, the cerebellum is one of the first structures to differentiate however; it achieves its mature configuration after birth. Sadler (2012[2]), described that the dorsolateral parts of the alar plates bend toward the midline and form the rhombic lips. In the caudal portion of the metencephalon, the rhombic lips are separated, but immediately below the mesencephalon, they approach medially. As a result of deepening of the pontine flexure, the rhombic lips compress cephalocaudally and form the cerebellar plate. Therefore, the cerebellum is liable to developmental irregularities.

Cisplatin is one of the most efficient anti-cancer drugs that are used in the treatment of many kinds of tumors. The toxic effects of cisplatin is mainly posed on the cells of nervous tissues, in the studies which have been done in this field so far, and the teratogenic effects of the drug on embryo and its malformation have been proven. The cells of cerebellar tissue are examples of cells which

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are sensitive to this drug. Cisplatin treatment increases the level of free oxygen radicals in the neuronal tissue[3].

Several studies have shown that the alpha lipoic acid causes neuro protective effects against chemotherapy-induced neurotoxicity by preventing axonal damage, and mitochondrial dysfunction in the nerve cells of the central nervous system[4].

Alpha "α" lipoic acid presents in the mitochondrial matrix in the cells of organisms where cells metabolisms and energy production take place. Lipoic acid presents in the reduced formula in living organisms, and catalyzes oxidative decarboxylation process causing change pyruvate to acetyl CoA. It is an important cofactor in energy-producing pathways and its antioxidant activity appears by increasing the activity of enzymes that protect against reactive oxygen species and by reducing oxidative stress[5]. In addition, ALA appears its antioxidant effects by increasing the glutathione peroxidase activity.

AIM OF THE WORK

The aim of the present work is to study the development of the cerebellum and the effect of cisplatin on its development. In addition the possible protective role of alpha lipoic acid has been investigated through cytoarchitectonic, ultrastructural, immunohistochemical and morphometric study.

MATERIAL AND METHODS

A total number of 40 pregnant female albino rats were used in this work. Animals were obtained from the animal house of Assiut University. They were reared under standard laboratory conditions with free access to food and water. Separation between adult male and female rats were done for 20 days to sure that the female were not pregnant. After this , mating was allowed between the male and female rats. All the females showing the vaginal plug were considered to be in the first day of pregnancy. The pregnant female rats were classified into 4 groups:

1- Group (I): It was considered as (control group) and they received no treatment.

2- Group (II) (cisplatin treated group): Animals were treated with cisplatin at a dose of 4 mg/kg body weight once weekly by intra peritoneal injection started from the sixth day of pregnancy and continued till the end of lactation period of rat. The drug was freshly dissolved in sterile saline immediately before injection[6].

3- Group (III) (alpha lipoic acid treated group): Animals were treated with α lipoic acid at a dose of 100 mg/kg daily by intra peritoneal injection started from the beginning of pregnancy till end of lactation[7].

4- Group (IV) (both alpha lipoic acid and cisplatin received group): This group was treated with both cisplatin and alpha lipoic acid. The animals were received both cisplatin at the sixth day of pregnancy and alpha lipoic acid at the beginning of pregnancy using the same doses which given to groups (II) and (III).

The animals were weighted and received the calculated dose of the drug according to their weight. At the end of the experiment, the offsprings were sacrificed. The following age groups were studied in the control and experimental animals (newborn, 10 days and 20 days rats). The offsprings were sacrificed and the brains were extracted from the skulls and the cerebella were dissected.

In all the studied groups 6 cerebella were processed to be studied by light microscopic study using Einason´s gallocyanine stain according to the steps described by (Bancroft[8]). For electron microscopic study, specimens from 6 cerebella in all the studied groups were fixed in phosphate buffered gluteraldhyde for 24 hours and post fixed in 1% osmium tetraoxide for one hour. Semithin sections (1μ) were prepared and stained with toluidine blue. Ultrathin sections (450-500A) from selected areas were contrasted with uranyle acetate and lead citrate and photographed in Assiut University Electron Microscopic Unit.

Immunohistochemical study using glial fibrillary acidic protein (GFAP) was done on 6 cerebella in each of the studied groups. The steps were done according (Cattoretti[9]).

Morphometric procedure: In this work, estimation of the number of purkinje cells and

granule cells in the internal granular layer per area 12360 μ² was done in all the studied groups. It was done by using Image Analyzer soft imaging system (Olympus company) in faculty of medicine at Assiut university. Data were presented as mean±SD. Statistical analysis of data was tested for significance using the student t-test through the computerized statistical package "Prism".

Finally, significance was considered according to the *p - value* level of significance as follows according to (Durkee[10]):

p>0.05 (NS) non significant. *p<0.05* significant. *p<0.01* highly significant.

RESULTS

1- Newborn rat:

The cerebellum of control group is composed of cerebellar cortex and white matter containing deep cerebellar nuclei (Fig. 1).

In new born, the cerebellar cortex was found to be formed of four layers.

The external granular layer is characteristic feature of the developing cerebellum. It appears to be formed of tightly packed small neurons which divided into two zones:

1- The multiplying zone (MZ) which situated beneath the pia matter. The cells of MZ have rounded outline with prominent nucleus and nucleolus

2- The premigratory zone (PZ) is located deeper to the multiplying zone (MZ). Its neurons will migrate to the internal granular layer.

The second layer is the molecular layer. This layer is composed of few scattered cells. In addition, it demonstrated the presence of columns of migrating neurons.

The purkinje cells appear to be arranged into several layers. Two types of purkinje cells can be observed. Some of cells are darkly stained with rounded nuclei and prominent nucleoli while the other cells are lightly stained and more superficially located. The fourth layer is the internal granular layer whose cells are small and rounded with well defined nuclei (Fig. 2).

Electron microscopic study demonstrated that the purkinje cell has large nucleus with uniform chromatin distribution and prominent two nucleoli. The cytoplasm shows the presence of many ribosomes, rough endoplasmic reticulum and mitochondria (Fig. 6). Examination of presynaptic terminal making synaptic contact with purkinje cell soma revealed the presence of lot of synaptic vesicles and mitochondria.

The white matter of the cerebellum shows the presence of deep nuclei which situated beneath the floor of fourth ventricle. Its cells are closely packed. There are 2 types of cells, some large cells with oval nucleus and prominent nucleolus while other cells are small with darkly stained nuclei (Fig. 9).

In newly born rat treated with cisplatin, semithin sections shows that the external granular appeared to be more thick in comparison with control group. The molecular layer which is the second layer contains small scattered neurons. The purkinje cell appears to have vacuolated cytoplasm. The cells of internal granular layer in this group appeared to have vacuolated cytoplasm and darkly stained nuclei (Fig. 3).

By using electron microscopic technique, the nucleus of purkinje cell has irregular nuclear membrane. Its cytoplasm contains dense bodies and many vacuoles (Fig.7). As the regarding the synaptic contact with purkinje cell soma, there are extensive loss of synaptic vesicles and mitochondria. The neurons of deep cerebellar nuclei appear to be scattered with many cells have darkly stained nuclei (Fig. 10).

The cerebellar cortex in the group (III) appears to be similar to that of control (Fig. 4)

In the newly born rat of mothers treated with cisplatin and alpha lipoic acid the external granular seems to be relatively thicker than that of the control. Some of cells have vacuolated cytoplasm. The purkinje cell arranged in rows. The neurons of internal granular layer appear to be rounded with darkly stained nuclei (Fig. 5). Transmission electron microscopic study revealed that the purkinje cell has oval nucleus with uniform chromatin distribution and the cytoplasm contains free ribosomes, lot of mitochondria and rough endoplasmic reticulum (Fig. 8). The

presynaptic terminal had a lot of synaptic vesicles and mitochondria.

Examination of the cells of deep cerebellar nuclei revealed that there are many cells with large oval nuclei and prominent nuclei. There are some cells with darkly stained nuclei (Fig. 11).

Morphometric study: The mean number of purkinje cells in cisplatin received group is 10.11 ± 1.62 which shows significant decrease ($p<0.01$) as compared with control where the mean is 21.78±2.82. In the group (III) the mean number is 22.11±4.14 which shows insignificant difference as compared with control group. In group (IV), the mean number of purkinje cells is 17.67±3.81 which shows insignificant difference as compared with control.

The mean number of granule cells in cisplatin received group is 47.78±6.44 which shows significant decrease ($P<0.01$) as compared with control where the mean is. In the group (III) the mean number is 78.44±7.84 which shows insignificant difference as compared with control group. In group (IV), the mean number of granule cells is 72.44±7.13 which shows insignificant difference as compared with control.

2-Ten days old rat:

Light microscopic study of the developing cerebellar cortex at this age of control group shows the presence of external granular layer. The deep cells of external granular layer appeared to be dispersed in the molecular layer (Fig. 23). This feature in the cells of external granular layer is due to migration of neurons to their final position in the internal granular layer.

The molecular layer which is characterized by increase in its size as compared with age of newborn rat. This is due to appearance of inhibitory interneurons which are the basket cells and the stellate cells.

The purkinje cell layer is still arranged in several rows. The purkinje cell body is disc or pear shape containing apical cytoplasmic cone with vesicular nucleus. Some of purkinje cell bodies contain basal cytoplasm which representing a transitional stage of maturation. The internal granular layer contains neurons with rounded nuclei with condensed chromatin (Fig. 12).

Ultrastructural study of the purkinje cells shows that the cells have rounded purkinje nucleus and its cytoplasm contains mitochondria, many free ribosomes, rough endoplasmic reticulum and Golgi apparatus (Fig. 16). The presynaptic terminal making synaptic contact with the purkinje cell is characterized by the presence of many synaptic vesicles and mitochondria.

Immunohistochemical examination of the cerebellum using anti GFAP shows the presence of weak expression of anti GFAP in all layers of the cerebellum. Few immunoreactive fibers in the external granular layer and molecular layer appear to be present. Some neuroglial cells in purkinje cell layer and internal granular layer can be observed (Fig. 19).

Examination of the cerebellar white matter shows the presence of groups of cerebellar nuclei. These nuclei are classified into two main groups:

1- Caudal group that located in relation to fourth ventricle.

2- Rostral group located deeply in the cerebellar white matter.

The cells of the caudal group are formed mainly by medial cerebellar nucleus (fastigial nucleus). The cells are of variable size and shape. It is formed of small cells and large cells. The large cells appeared to have prominent nuclei which surrounded by dark cytoplasm which it is rich in Nissl granules. The neurons of the rostral group are formed mainly by lateral cerebellar nucleus (Dentate nucleus). It is divided into magnocellular part and parvocellular part. The neurons of dentate nucleus are fusiform with oval nucleus and prominent nucleolus and its cytoplasm is rich in Nissl granules. In addition, there are many scattered small cells with rounded nuclei (Fig. 23).

The cerebellar cortex, of cisplatin received group shows apparant increase in the distance between the pia matter and cerebellar cortex. It also shows that there is increase in the thickness of external granular layer in comparison with the control. The 2 zones (multiplying zone (MZ)

and premigratory zone (PZ)) of the external granular layer contain cells with rounded nuclei and prominent nucleoli. The PZ contains some cells with darkly stained nuclei.The purkinje cell layer appears to be arranged in several rows. Most of cells of purkinje layer are faintly stained. Some of them have vacuolated cytoplasm with darkly stained nuclei. Also, many cells of the internal granular layer have degenerative changes (Fig. 13)

The examination of purkinje cell with electron microscope revealed that the nucleus shows chromatin condensation with irregularity in the nuclear membrane. Its cytoplasm contains dilated rough endoplasmic reticulum and damaged mitochondria. There are marked loss of free ribosomes in comparison with control (Fig. 17). The presynaptic terminal making synaptic contact with the purkinje cells revealed that there are loss of synaptic vesicles and mitochondria.

Immunohistological study reveals that, there is apparent increase in immunoreactivity in all layers of the cortex as compared with control. A dense network of immunoreactive fibers is found to be present in the external granular layer and molecular layer. Also, many neuroglial cells with processes appear to be present in the purkinje layer and internal granular layer (Fig. 20).

Examination of deep nuclei showed that both rostral and caudal groups are lightly stained in comparison with the control. The cells of medial cerebellar nuclei appear to be widely dispersed. Many cells of these nuclei have vacuolated cytoplasm (Fig. 24a).

The cells of magnocellular part of dentate nucleus have lightly stained cytoplasm. The cells of parvocellular part are widely scattered. The neurons of both parts of dentate nucleus have vacuolated cytoplasm (Fig. 24 b).

Histological examination of group received alpha lipoic acid demonstrated the normal arrangement of the layers of the cerebellar cortex (Fig. 14).

In the rats of group (IV), the thickness of external granular layer appears nearly similar to that of control. The cells of external granular layer have regular outline with rounded nucleus and nucleolus. some of neurons showed the presence of darkly stained nuclei. The purkinje cell layer contains neurons which are large with rounded nuclei and with granular cytoplasm). Most of the cells of the internal granular layer have rounded nuclei with fine dispersed chromatin. However, some cells appear to have vacuolated cytoplasm (Fig. 15).

Ultrastructural study of purkinje cell demonstrated that the nucleus has evenly distributed chromatin and its cytoplasm contains mitochondria, rough endoplasmic reticulum and ribosomes. It is nearly comparable to that of control (Fig. 18). The presynaptic terminal making synaptic contact with purkinje cells appears to have synaptic vesicles nearly comparable to that of control.

Apparent decrease in the immunoreactive fibers and glial cells in all layers of cerebellum as compared with group received cisplatin only could be observed (Fig. 21).

Examination of the white matter of the cerebellum demonstrate that the neurons of rostral group and caudal group are closely packed in comparison to the group (II).

The cells of fastigial nucleus have regular outline with prominent nucleus and nucleolus. some of cells appear to have darkly stained nuclei with vacuolation of its cytoplasm (Fig. 25 a). Most of the cells of dentate nucleus have well defined outline with vesicular nucleus and prominent nucleolus. Few cells show the presence of darkly stained nuclei (Fig. 25 b).

The mean number of purkinje cells in cisplatin received group is 6.00±1.32 which shows significant decrease ($p<0.01$) as compared with control where the mean is 12.00±2.55. In the group (III) the mean number is 11.78±2.11which shows insignificant difference as compared with control group. In group (IV), the mean number of purkinje cells is 10.33±1.80 which shows insignificant difference as compared with control.

The mean number of granule cells in cisplatin received group is 283.56±33.99 which shows significant decrease ($p<0.01$) as compared with control where the mean is 347.33±41.07. In the group (III) the mean number is 339.11±41.65 which shows insignificant difference as compared with control group. In group (IV), the mean

number of granule cells is 302.67±65.90 which shows insignificant difference as compared with control.

3-Twenty days old rat:

The cerebellar cortex of control group is differentiated in to molecular layer, purkinje cell layer and granular layer. As the regarding the external granular layer, it is nearly disappeared with the exception of few cells can be identified. The molecular layer has few scattered cells. The purkinje cells are arranged in one row which is characteristic of its maturity. The granule cells in the internal granular layer appear to be arranged in clusters. The cells have well defined nuclei (Fig. 26).

On the examination of purkinje cells by electron microscope, its nucleus is oval in shape with uniformly distributed chromatin. The cytoplasm contains rough endoplasmic reticulum , mitochondria and free ribosomes (Fig. 30). The presynaptic terminal making synaptic contact with purkinje cell contains a lot of synaptic vesicles and mitochondria .

Immunoreactive cells and fibers appear to be scattered in the molecular layer, in the purkinje cell layer and granular cell layer (Fig. 33).

The cells of deep cerebellar nuclei appear to be more differentiated than previous age (Fig. 36).

Examination of rats of group (II) revealed that there is marked increase in the thickness of the external granular layer in comparison with that of control. The molecular layer shows the presence of columns of migratory cells. Some of neurons have darkly stained nuclei. Many of purkinje cells have darkly stained nuclei. The granule cells in the internal granular layer have condensed chromatin (Fig. 27). Electron microscopic study reveals that the nucleus of purkinje cells has peripheral chromatin condensation. The cytoplasm contains many vacuoles, dense bodies, dilated rough endoplasmic reticulum and marked loss of free ribosomes (Fig. 31). The presynaptic terminal which making synaptic contact with the purkinje cell demonstrates marked loss of synaptic vesicles and damaged mitochondria.

All layers of the cerebellar cortex showed intense immunostaining with extensive network of fibers and many glial cells in purkinje cell layer and granular cell layer (Fig. 34).

Cells of fastigial nucleus appeared to have darkly stained nuclei while other cells have vacuolated cytoplasm (Fig. 37a). Neurons of dentate nucleus are widely dispersed and lightly stained due to reduction of Nissl granules (Fig. 37 b).

The cerebellar cortex of group (III) is nearly comparable to that of the control (Fig. 28).

The rats of group (IV) reveals the presence of external granular layer which is formed of many rows of cells. The molecular layer has many cells with darkly stained nuclei. The neurons of purkinje cell layer have oval nuclei with prominent nucleolus.Some of cells have darkly stained nuclei. The granule cells in the internal granular layer arrange in clusters. They have rounded nuclei with prominent nucleoli. Some cells have darkly stained nuclei (Fig. 29).

Electron microscopic examination revealed that the nucleus of purkinje cell has fine evenly distributed granular chromatin which is equally distributed. The cytoplasm contains a lot of free ribosomes and mitochondria. There are some vacuoles in the cytoplasm. The presynaptic terminal making synaptic contact with purkinje cell shows the presence of synaptic vesicles and mitochondria (Fig. 32).

Moderate amount of GFAP fibers and cells with long processes could be observed in the purkinje layer and internal granular layer (Fig. 35).

Most of the cells of fastigial and dentate nuclei have normal appearance. Only few cells with darkly stained nuclei and vacuolated cytoplasm could be observed (Fig. 38).

The mean number of purkinje cells in cisplatin received group is 3.22±0.83 which shows significant decrease ($p<0.01$) as compared with control where the mean is 5.00±1.12. In the group (III) the mean number is 4.44±1.94 which shows insignificant difference as compared with control group. In group (IV), the mean number of purkinje

cells is 5.33±1.00 which shows insignificant difference as compared with control.

The mean number of granule cells in cisplatin received group is 221.56±6.58 which shows significant decrease ($p<0.01$) as compared with control where the mean is 412.33±7.60. In the group (III) the mean number is 408.89±22.62 which shows insignificant difference as compared with control group. In group (IV), the mean number of granule cells is 392.22±13.81 which shows insignificant difference as compared with control.

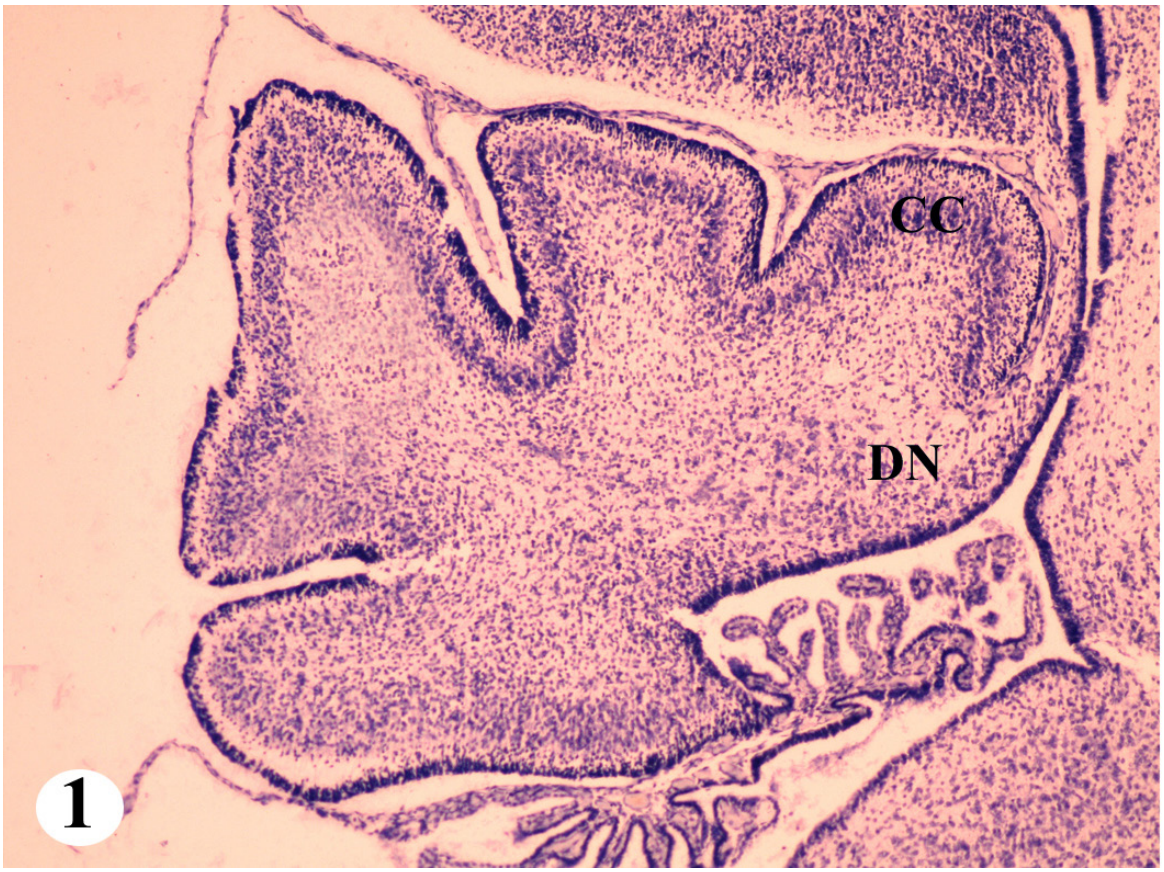


Plate 1:

Fig. 1: A photomicrograph showing sagittal section of the cerebellum of newborn rat offspring of control mother. It shows the different layers of the cerebellar cortex (CC). Note the presence of deep cerebellar nuclei (DN) in the white matter of the cerebellum. (Gallocyanine stain ×100).

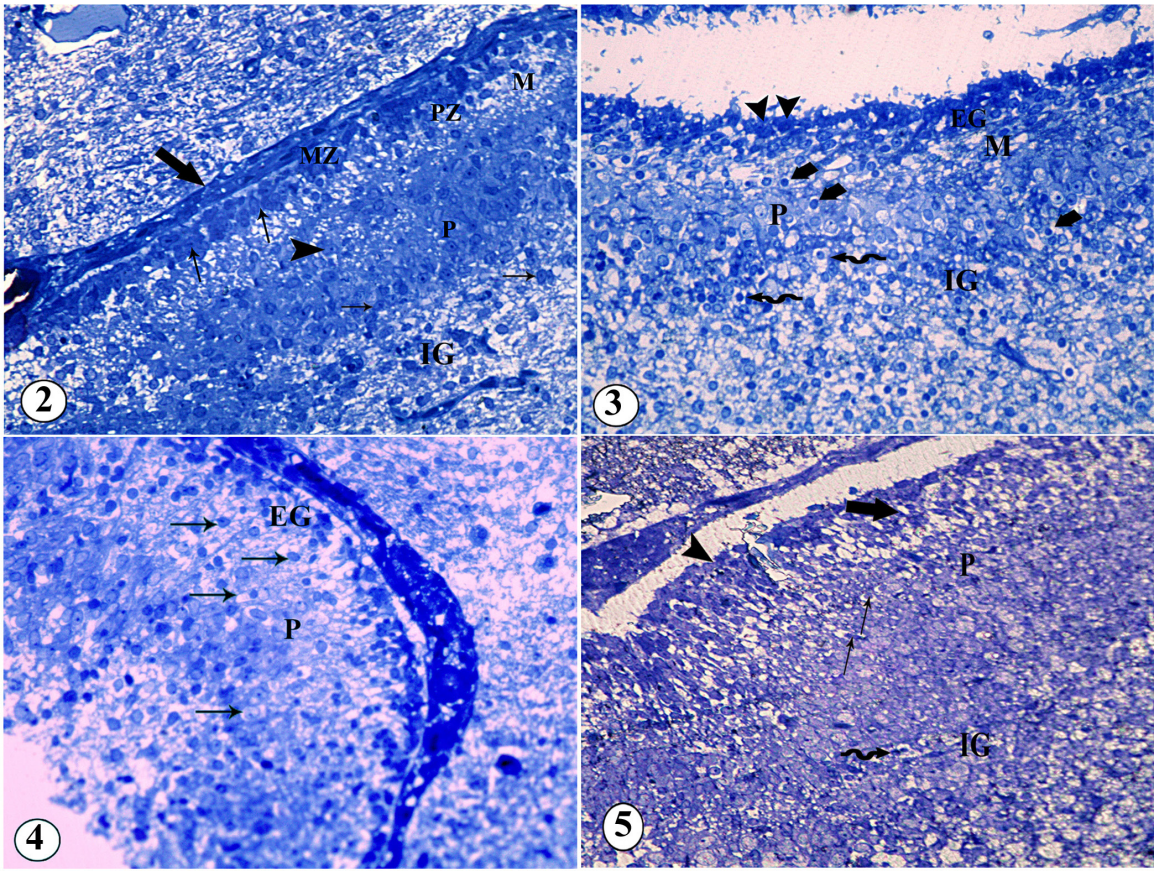


Plate 2:

Fig. 2: A photomicrograph of the cerebellar cortex of newly born rat of control group (I) showing the external granular layer which is composed of 2 zones multiplying zone (MZ) just deep to the pia matter (thick arrow) and premigratory zone (PZ) deeper to multiplying zone. The cells of (MZ) have rounded outline with prominent nucleus and nucleolus (arrow). The molecular layer (M) has scattered cells. The purkinje cells arranged in multi layers (P). The purkinje cells show the presence of two types of cells. Some of the cells are darkly stained with rounded nucleus with prominent nucleolus (arrow). The other cells are lightly stained and located more superficially (arrow head). The internal granular layer shows the presence of small rounded cells with well defined nucleus (arrow). (Toluidine blue stain ×400).

Fig (3): A photomicrograph showing the cerebellar cortex of newly born rat whose mother received cisplatin (II). The external granular layer (EG) appears to be more thick in comparison with that of the control (arrow head). The molecular layer (M) has scattered cells. Some of the purkinje cells have vacuolated cytoplasm (Thick arrow).The internal granular layer (IG) has many cells with vacuolated cytoplasm and darkly stained nuclei (wavy arrow). (Toludine blue stain ×400).

Fig.(4): A photomicrograph showing the cerebellar cortex of newly born rat whose mother received alpha lipoic acid only (III). The external granular layer (EG) contain the cells that migrate to reach the internal granular layer (arrow). The purkinje cell layer (P) arranged in multi layers. The cells of purkinje cells have regular outline (arrow). (Toluidine blue ×400).

Fig. (5): A photomicrograph of cerebellar cortex of newly born rat whose mother received alpha lipoic acid and cisplatin (group IV). The external granular layer appears relatively thicker than that of control (arrow head). Some of cells have vacuolated cytoplasm (Thick arrow). The purkinje cell layer (P) is formed of several rows of cells similar to that of control. Few cells have vacuolated cytoplasm and darkly stained nuclei (arrow). The cells of internal granular layer are rounded with darkly stained nuclei (wavy arrow). (Toluidine blue×400).

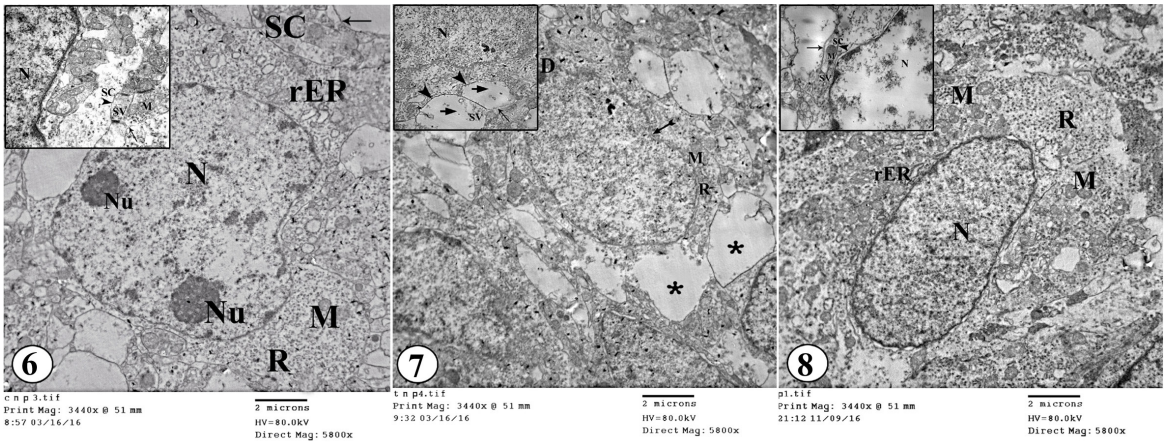


Plate 3:

Fig. 6: An electron photomicrograph showing the purkinje cell of newborn rat offspring of control mother. The cell has large nucleus (N) with uniformly distributed chromatin and prominent 2 nucleoli (NU). The cytoplasm shows the presence of many free ribosomes (R), rough endoplasmic reticulum (rER) and mitochondria (M). Note the presence of many presynaptic terminals(arrow) making synaptic contact (SC) with body of the cell. (×5800).

Inset: shows the synaptic contact (SC) (arrow head) of presynaptic terminals (arrow) with the purkinje cell of the cerebellar cortex of new born rat offspring of control mother. Note the presence of many synaptic vesicles (SV) and mitochondria (M) in the presynaptic terminals. N is for purkinje cell nucleus. (×14000).

Fig. 7: An electron photomicrograph showing the purkinje cell of newborn rat offspring of mother recieved cisplatin. The nucleus has irregular nuclear membrane (tailed arrow) with condensation of chromatin. The cytoplasm contains free ribosomes (R) and mitochondria (M). Note the presence of dense bodies (D) and many vacuoles of variable sizes (stars). (×5800).

Inset : shows synaptic contact (arrow head) between presynaptic terminals (arrow) and purkinje cell offspring of mother treated with cisplatin. Note the extensive loss of synaptic vesicles and mitochondria in presynaptic terminals (short arrow). SV is for synaptic vesicles and N is for nucleus of purkinje cell. (×14000).

Fig. 8: An electron photomicrograph of purkinje cell of newly born rat offspring of mother treated with alpha lipoic acid and cisplatin. The nucleus (N) is oval shaped with uniformly distributed chromatin. The cytoplasm is filled with free ribosomes (R) , rough endoplasmic reticulum (rER) and a lot of mitochondria (M). (×5800).

Inset : shows synaptic contact (arrow head) between presynaptic terminal (arrow) and purkinje cell offspring of mother treated with cisplatin and alpha lipoic acid. The presynaptic terminals have a lot of synaptic vesicles (SV) and mitochondria (M). Note that it appears relatively similar to that of control. Note the presence of purkinje cell nucleus (N). (×14000).

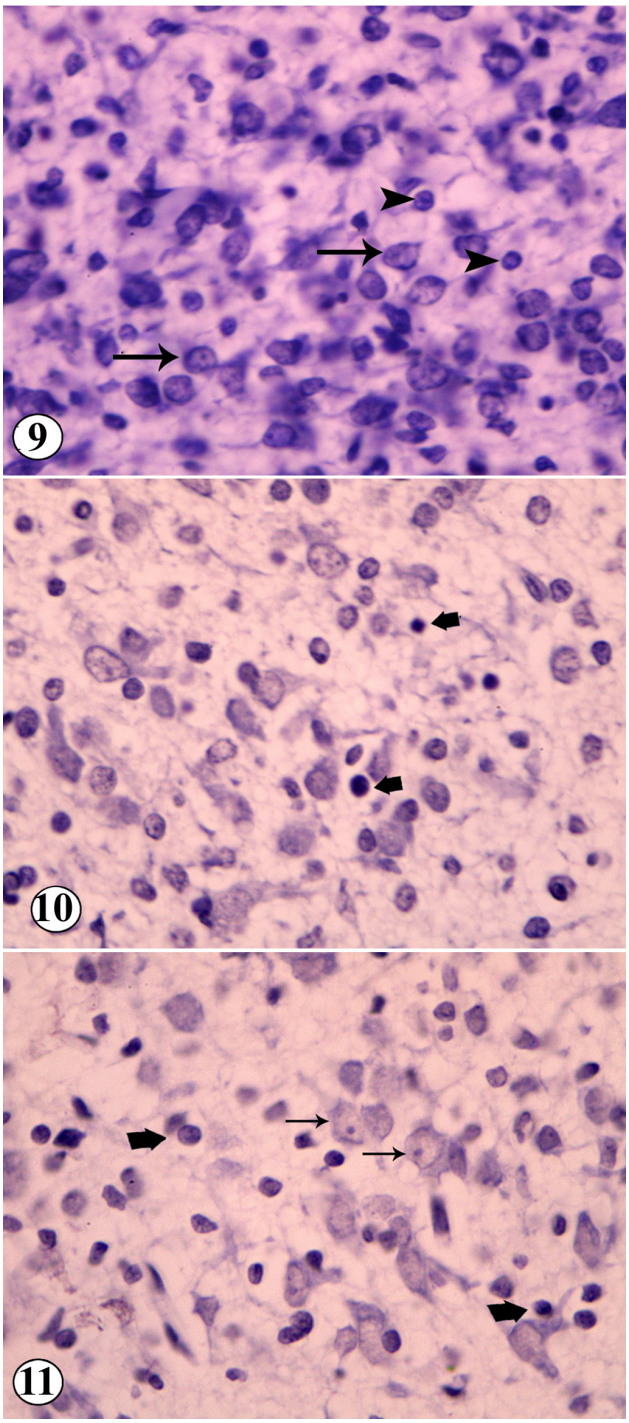


Plate 4:

Fig. 9: A photomicrograph of the region of deep cerebellar nuclei in the cerebellum of new born rat offspring of control mother. The cells are of different shape and size. Some cells are large with oval nucleus and prominent nucleolus (arrow). Other cells are small with darkly stained nuclei (arrow head). (Gallocyanine stain×1000).

Fig. 10: A photomicrograph of the cerebellum of new born rat offspring of mother received cisplatin. The cells of deep cerebellar nuclei are widely dispersed and lightly stained. Note the presence of many cells with darkly stained nuclei (thick arrow). (Gallocyanine stain×1000).

Fig. 11: A photomicrograph of the cerebellum of new born rat offspring of mother received alpha lipoic acid and cisplatin in the region of deep cerebellar nuclei. It shows the presence of cells with large oval nuclei and prominent nuclei (arrow). The surrounding cytoplasm appears to be filled with Nissl granules. Note the presence of some cells with darkly stained nuclei (Thick arrow). (Gallocyanine stain×1000).

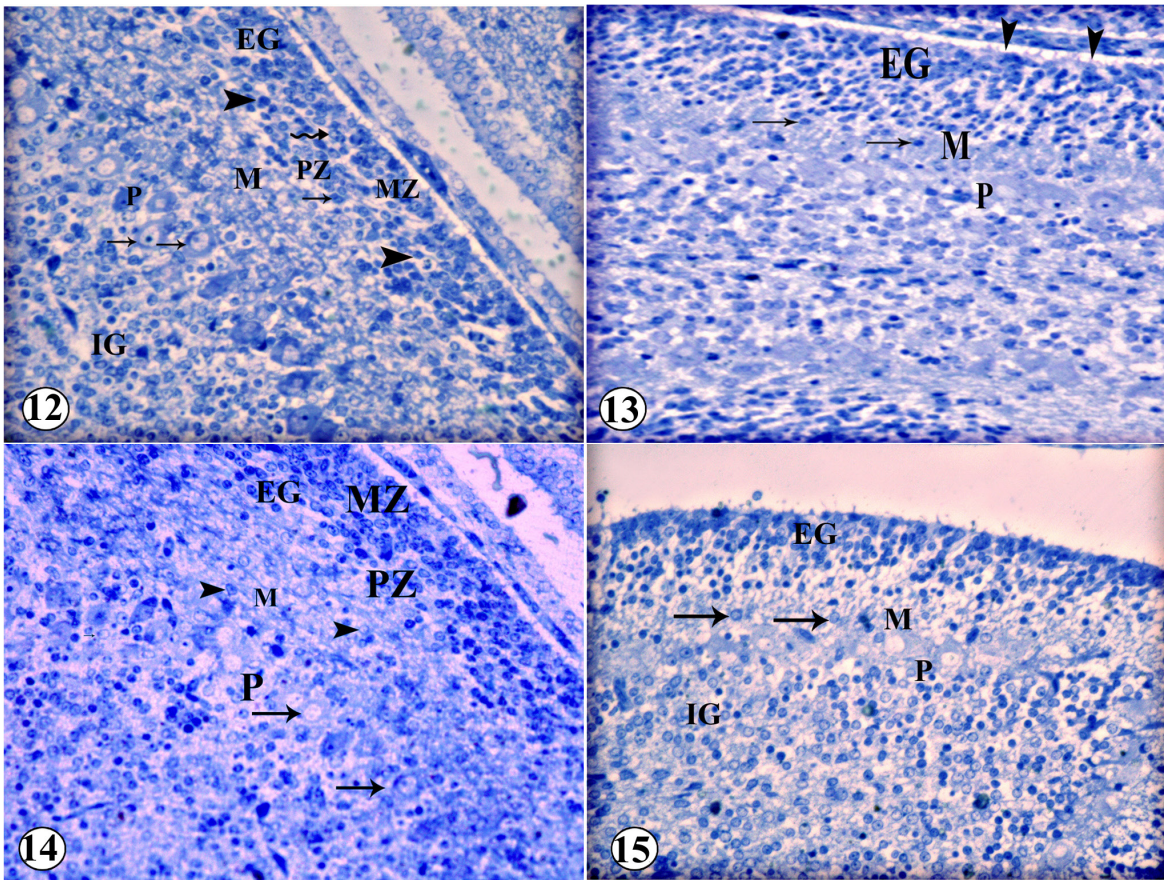


Plate 5:

Fig. 12: A photomicrograph of the cerebellar cortex of 10 days old rat of control group showing differentiation of external granular layer (EG) into two zones which are multiplying zone (MZ) and premigratory zone (PZ). The cells of MZ are regular in outline with nucleus . Some cells have two nucleoli (wavy arrow). Cells of PZ showed well defined nucleus and nucleolus (arrow). Some cells have darkly stained nuclei (arrow head). The molecular layer (M) shows the presence of many scattered cells. The purkinje cell layer (P) appears to be arranged in more than one row(arrow). The internal granular layer (IG) have many rounded cells with prominent nucleus and nucleolus. (Toluidine blue stain ×400).

Fig 13: A photomicrograph showing the cerebellar cortex of ten days rat whose mother received cisplatin. There is apparent increase in the distance between pia matter and cerebellar cortex (arrow head). The external granular layer (EG) shows increase in its thickness in comparison with of the control. The molecular layer (M) shows the presence of many migratory cells (arrow). The purkinje cell layer (P) appears to be arranged in several rows. Some of cells of the internal granular layer have darkly stained nuclei and vacuolated cytoplasm. (Toluidine blue stain ×400).

Fig. 14: A photomicrograph of cerebellar cortex in 10 days old rats of mother which received alpha lipoic acid only. The external granular layer (EG) is differentiated into 2 zones : multiplying zone (MZ) and premigratory zone (PZ). The molecular (M) layer contain columns of migratory cells (arrow head). The purkinje cells (P)arranged in many rows (arrow). (Toluidine blue stain ×400).

Fig. 15: A photomicrograph of cerebellar cortex of 10 days old rat whose mother received alpha lipoic acid and cisplatin. It shows that the thickness of the external granular layer (EG) is nearly similar to that of control. Many columns of migratory cells can be observed in the molecular layer (M) (arrow). The purkinje cell layer (P) and the internal granular layer (IG) are nearly similar to those of the control (Toluidine blue×400).

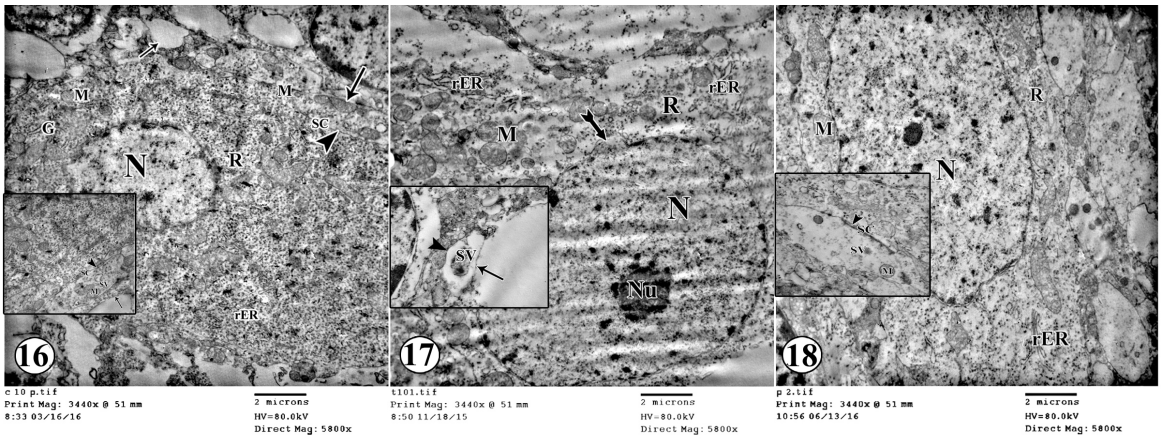


Plate 6:

Fig. 16: An electron photomicrograph showing the purkinje cell of ten days old rat offspring of control mother. The cell has a rounded euchromatic nucleus (N). The cytoplasm contains many free ribosomes (R), mitochondria (M), rough endoplasmic reticulum (rER) and Golgi apparatus (G). Note the presence of many presynaptic terminals (arrow) making synaptic contact (SC) (arrow head) with the soma of the neuron. (×5800).

Inset: shows presynaptic terminal (arrow) making synaptic contact (arrow head) with purkinje cell in the cerebellar cortex of ten days old rat offspring of control mother. The presynaptic terminal contains a lot of synaptic vesicles (SV) and mitochondria (M). (×14000).

Fig. 17: An electron photomicrograph showing the purkinje cell of ten days old rat offspring of mother recieved cisplatin. The nucleus (N) shows chromatin condensation and irregularity of nuclear membrane (tailed arrow). It has prominent nucleolus. The cytoplasm contains damaged mitochondria (M) and dilated rough endoplasmic reticulum (rER). Note marked loss of free ribosomes (R) (×5800).

Inset: shows synaptic contact (arrow head) on the purkinje cell of the cerebellar cortex of ten days old rat offspring of mother received cisplatin. Note the apparent decrease in the synaptic vesicles (SV) in the presynaptic terminal (arrow) which making synaptic contact (arrow head) with the soma of the neuron. (×14000).

Fig. 18: An electron photomicrograph of purkinje cell of ten days old rat offspring of mother treated with alpha lipoic acid and cisplatin. It shows that the nucleus has equally distributed chromatin. The cytoplasm contains a lot of free ribosomes (R), rough endoplasmic reticulum (rER) and mitochondria (M). It is nearly comparable to that of the control. (×5800).

Inset: shows synaptic contacts (arrow head) with the purkinje cell of the cerebellar cortex of ten days old rat offspring of mother received alpha lipoic acid and cisplatin. The presynaptic terminal contains synaptic vesicles (SV) and mitochondria (M). Note that the amount of synaptic vesicles is nearly comparable to that of the control. (×14000).

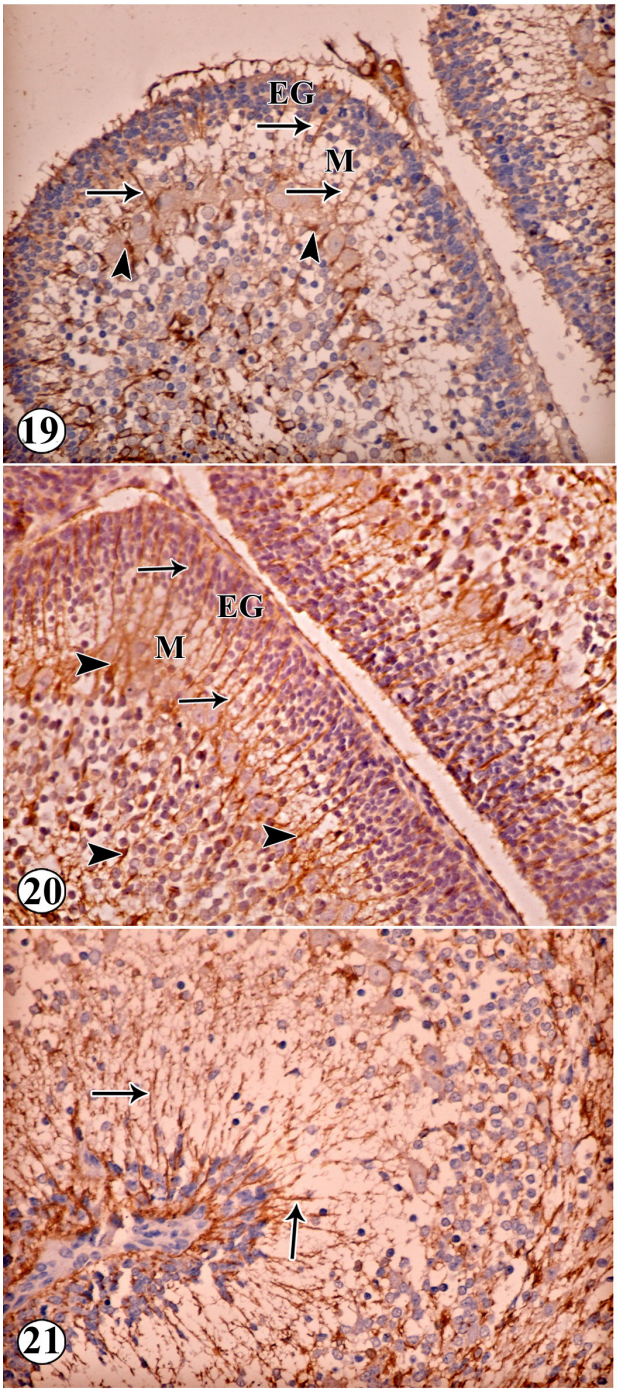


Plate 7:

Fig. 19: A photomicrograph showing the cerebellum of ten days old rat offspring of control mother. It shows the presence of immunoreactive fibers (arrow) in the in the external granular layer (EG) and molecular layer (M). Note the presence of neuroglial cells in between the soma of purkinje cells and in the internal granular layer (arrow head). (Anti GFAP immunostaining ×400).

Fig. 20: A photomicrograph showing the cerebellum of ten days old rat offspring of mother received cisplatin. It shows the presence of immunoreactive cells and extensive network of immunoreactive fibers (arrow) in the external granular layer (EG) and molecular layer (M).A lot of glial cells (arrow head) with their processes appear to be present in the purkinje and internal granular layer. (Anti GFAP immunostaining ×400).

Fig. 21: A photomicrograph showing the cerebellum of ten days old rat offspring of mother received alpha lipoic acid and cisplatin. It shows apparent decrease in the immunoreactive glial cells and fibers (arrow) as compared with group received cisplatin. (Anti GFAP immunostaining ×400).

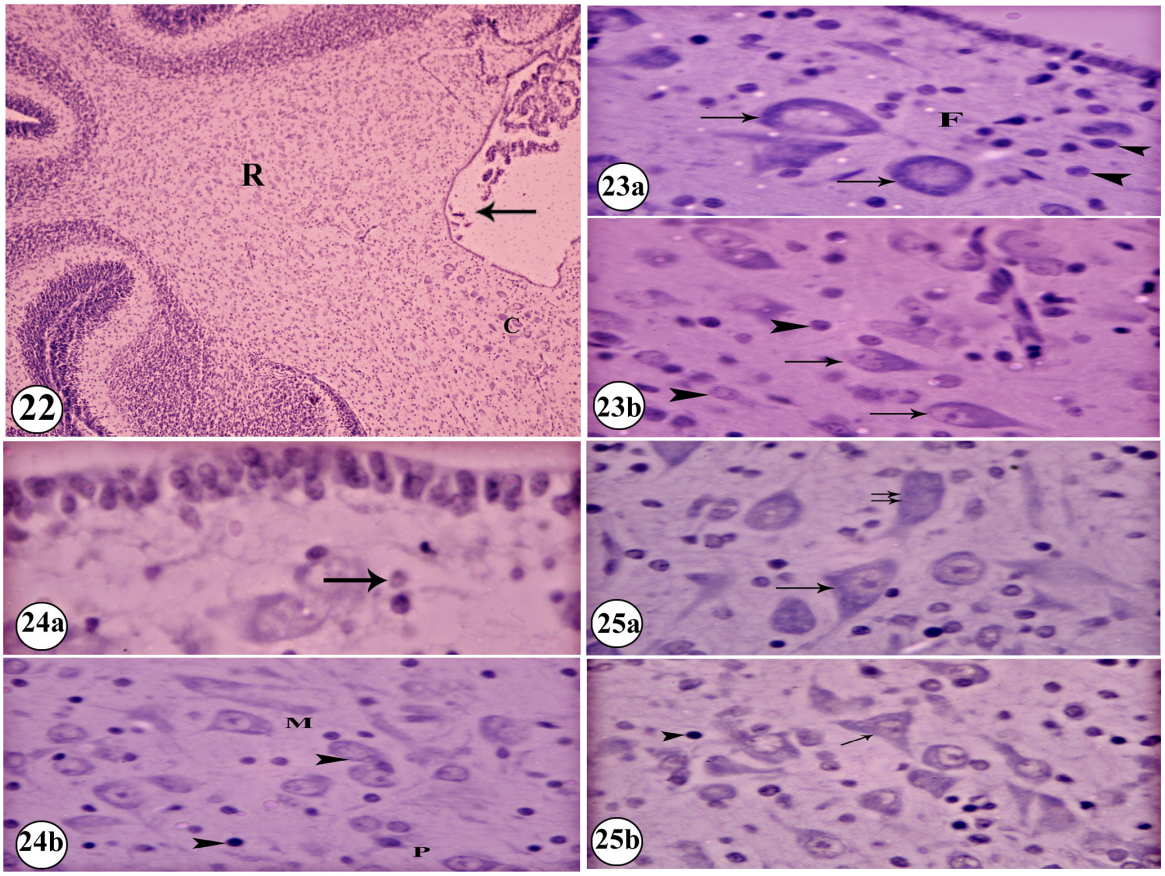


Plate 8:

Fig. 22: A photomicrograph showing the sagittal section of the cerebellum of ten days old rat offspring of control mother. It shows both caudal groups (C) of cerebellar nuclei in relation to fourth ventricle (arrow) and rostral group (R) in the white matter of the cerebellum. (Gallocyanine stain ×100).

Fig. 23 a: A photomicrograph showing the cerebellum of ten days old rat offspring of control mother. It shows that the medial cerebellar nucleus (fastigial)(F). It is formed of small cells (arrow head) and large cells (arrow). The large cells are variable in shape. They appear oval, rounded or star shape. They have prominent nuclei which surrounded by dark cytoplasm as it is rich in Nissl granules. (Gallocyanine stain×1000).

Fig. 23 b: A photomicrograph showing the cerebellum of ten days old rat offspring of control mother. It shows that the lateral cerebellar nucleus is composed of many large fusiform cells which have oval nucleus with prominent nucleolus. The cytoplasm is rich in Nissl granules (arrow). It also shows the presence of many cells with rounded nuclei (arrow head). (Gallocyanine stain×1000).

Fig. 24 a: A photomicrograph showing the cerebellum of ten days old rat offspring of mother received cisplatin. It shows that the medial cerebellar (fastigial) nucleus has many cells with vacuolated cytoplasm (arrow). (Gallocyanine stain×1000).

Fig. 24 b: A photomicrograph showing the cerebellum of ten days old rat offspring of mother which received cisplatin. It shows that many cells of the magnocellular part (M) and parvocellular part (P) of the lateral cerebellar nucleus (Dentate) nucleus have vacuolated cytoplasm (arrow head). (Gallocyanine stain×1000).

Fig. 25 a: A photomicrograph showing the cerebellum of ten days old rat offspring of mother received alpha lipoic acid and cisplatin. The cells of the fastigial nuclei are of variable shape with vesicular nuclei (arrow). Some cells are lightly stained and have ill defined nuclei (double arrow). (Gallocyanine stain×1000).

Fig. 25 b: A photomicrograph showing the cerebellum of ten days old rat offspring of mother which received alpha lipoic acid and cisplatin. The cells in the dentate nucleus seem to be like control cells in which it have vesicular nucleus and prominent nucleolus (arrow). It is nearly similar to that of control. Some of the cells have darkly stained nuclei (arrow head). (Gallocyanine stain×1000).

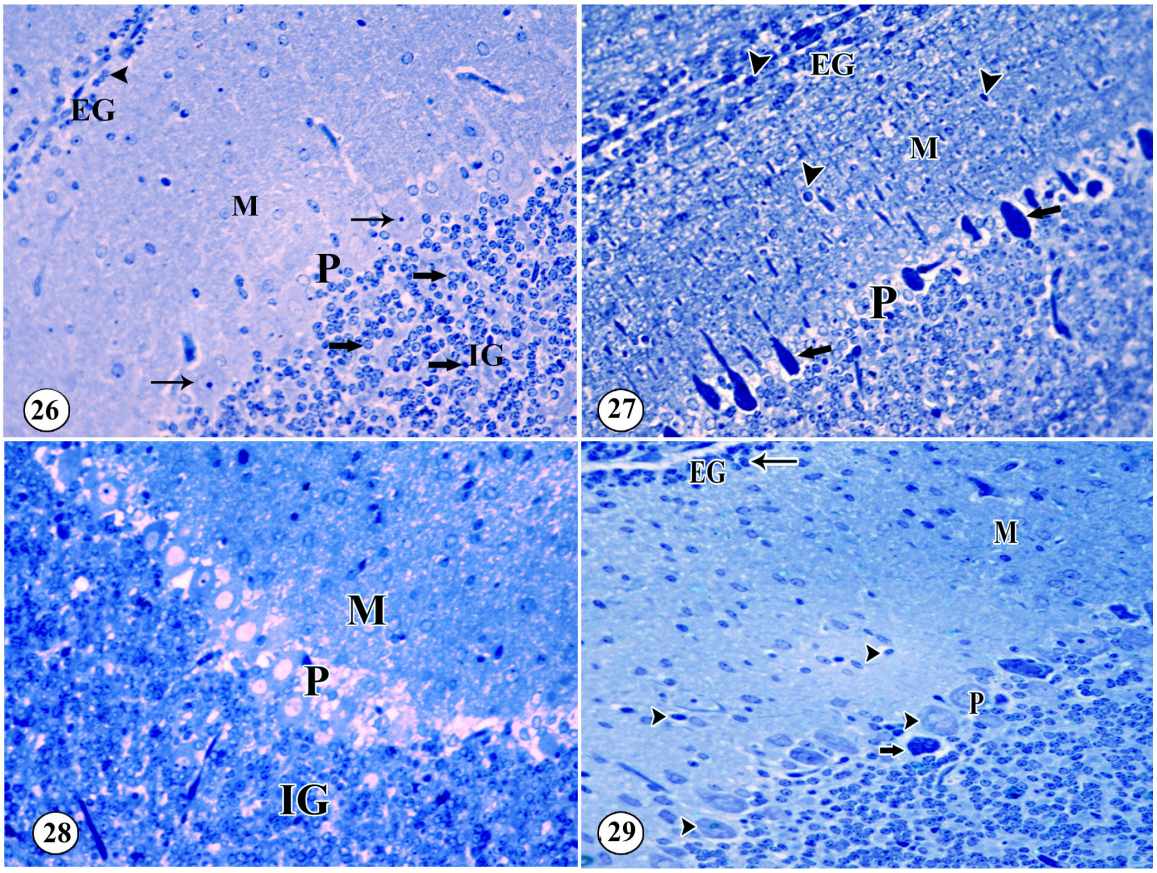


Plate 9:

Fig. 26: A photomicrograph showing the cerebellar cortex of twenty days old rat whose mothers of control group. It shows that the external granular layer (EG) is nearly disappeared. Only few cells can be observed (arrow head). The molecular layer (M) has few scattered cells. The purkinje (P)cells (arrow) are arranged in one row. The internal granular layer (IG) is formed of small rounded cells arranged in clusters (thick arrow). (Toluidine blue stain ×400).

Fig. 27: A photomicrograph showing the cerebellar cortex of twenty days old rat whose mothers received cisplatin. It shows marked increase in thickness of the external granular layer (EG) in comparison with control (arrow head). The molecular layer (M) has several cells with darkly stained nuclei (arrow head). Many of purkinje cells (P) have vacuolated cytoplasm and darkly stained nuclei (thick arrow). Note that they arranged in single row. The cells of the internal granular layer have darkly stained nuclei. (Toluidine blue stain ×400).

Fig. 28: A photomicrograph showing the cerebellar cortex of twenty days old rat whose mothers received alpha lipoic acid only. The cerebellar cortex is differentiated into molecular layer (M), purkinje cell layer (P) and internal granular layer (IG). (Toluidine blue stain ×400).

Fig. 29: A photomicrograph showing the cerebellar cortex of twenty days old rat whose mothers received alpha lipoic acid and cisplatin. The external granular layer (EG) appears to be formed of many rows of cells (arrow). The molecular layer (M) has many cells with dark nuclei (arrow head). The purkinje cells (P) arranged in one row of cells (arrow head). Note the presence of some cells with darkly stained nuclei (thick arrow). Cells of the internal granular layer are small and rounded. (Toluidine blue stain ×400).

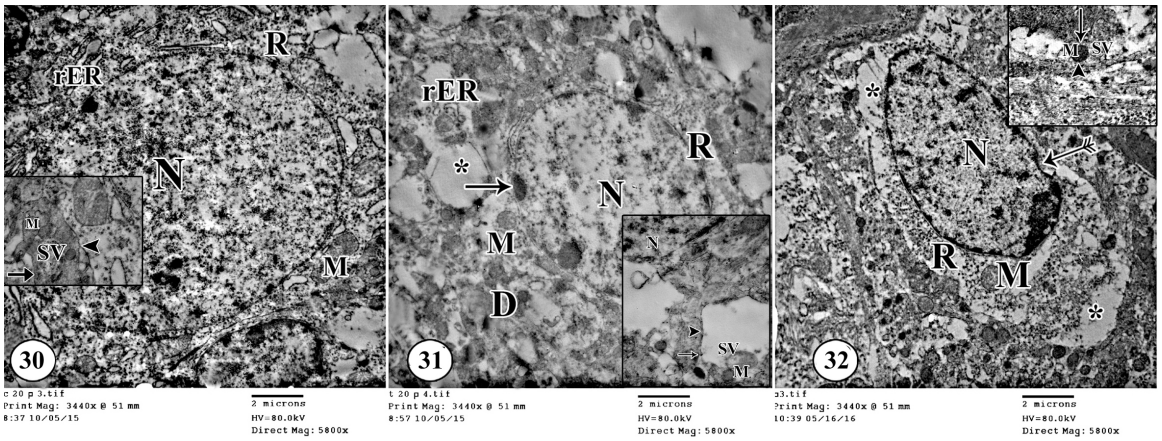


Plate 10:

Fig. 30: An electron photomicrograph of purkinje cell of cerebellar cortex of twenty days old rat offspring of control mother. The nucleus (N) is oval in shape with uniformly distributed fine granular chromatin. The cytoplasm contains mitochondria (M) and rough endoplasmic reticulum (rER) and a lot of free ribosomes (R). (×5800).

Inset: shows synaptic contact (arrow head) on the surface of purkinje cell in the cerebellum of twenty days old rat offspring of control mother. Note that the presynaptic terminal (arrow) contains synaptic vesicles(SV) of variable size and mitochondria (M). (×19000).

Fig. (31): An electron photomicrograph of purkinje cell of cerebellar cortex of twenty days old rat whose mother received cisplatin. The nucleus (N) has a peripheral chromatin condensation (arrow). The cytoplasm shows the presence of many vacuoles (stars), dense bodies (D), mitochondria (M) and dilated rough endoplasmic cisternae (rER). Note marked loss of free ribosomes (R). (×5800).

Inset: shows synaptic contact (arrow head) on the surface of purkinje cell in the cerebellum of twenty days old rat offspring of mothers received cisplatin. The presynaptic terminal (arrow) has marked loss of synaptic vesicles (SV) and shows the presence of damaged mitochondria (M). (N is for purkinje cell nucleus). (×19000).

Fig. 32: An electron photomicrograph of purkinje cell of cerebellar cortex of twenty days old rat whose mother received alpha lipoic acid and cisplatin. It shows that the nucleus (N) has fine granular evenly distributed chromatin. Double tailed arrow points for identation in the nuclear membrane. The cytoplasm contains ribosomes (R) and mitochondria (M). Note the presence of some vacuoles in the cytoplasm (star) (×5800).

Inset: shows synaptic contact (arrow head) on the surface of purkinje cell in the cerebellum of twenty days old rat offspring of mothers received alpha lipoic acid and cisplatin. The presynaptic terminal shows the presence of synaptic vesicles (SV) and mitochondria (M). (×14000).

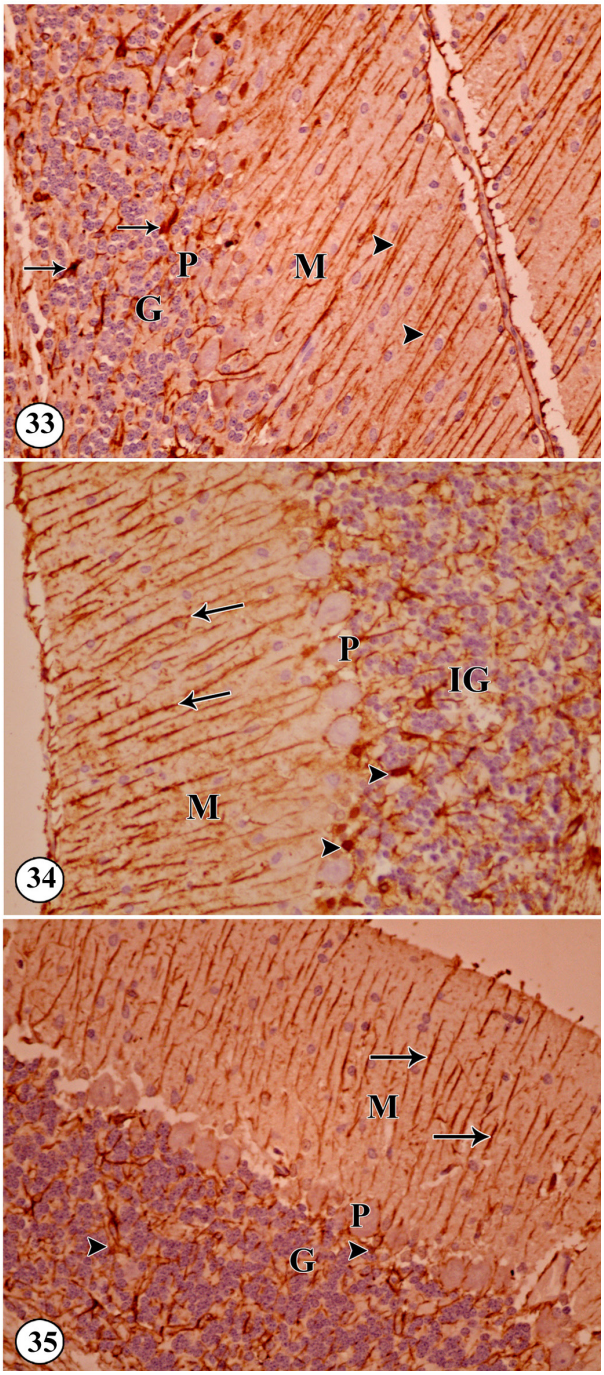


Plate11:

Fig. 33: A photomicrograph showing the cerebellum of twenty days old rat offspring of control mother. The molecular layer (M) shows the presence of immunoreactive cells and fibers (arrow head). some of the glial cells (arrow) are observed to be scattered among the purkinje (P) and granule cells (G). (Anti GFAP immunostaining×400).

Fig. 34: A photomicrograph showing the cerebellum of twenty days old rat offspring of mother treated with cisplatin. The molecular layer (M), shows extensive immunoreactive network of fibers (arrow). Many glial cells (arrow head) and their processes appear to present among the purkinje cells (P) and internal granular layer (IG). (Anti GFAP Immunostaining ×400).

Fig. 35: A photomicrograph showing the cerebellum of twenty days old rat offspring of mother treated with alpha lipoic acid and cisplatin. The molecular layer (M) shows the presence of some immunoreactive fibers (arrow). Some of glial cells (arrow head) and their processes are found to be scattered among the purkinje cells (P) and internal granular layer (G). Note the apparent decrease in immunoreactivity in comparison to group which received cisplatin only. (Anti GFAP Immunostaining ×400).

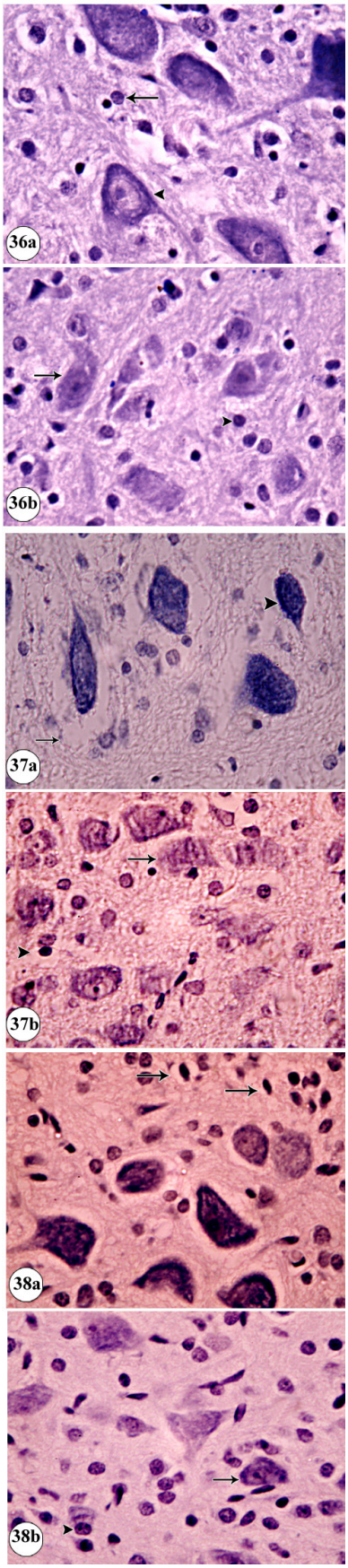


Plate 12:

Fig. 36 a: A photomicrograph of the cerebellum of twenty days old rat offspring of control mother. It shows that the small cells of the fastigial nucleus have rounded well defined nucleus(arrow). The large cells are oval or fusiform in shape They have oval nucleus with prominent nucleolus (arrow head). Their cytoplasm is rich with Nissl granules.

Fig. 36 b: A photomicrograph of the cerebellum of twenty days old rat offspring of control mother. It shows that most of the cells of magnocellular part of the dentate nucleus are fusiform in shape (arrow). They have large oval shaped nucleus with prominent nucleolus. Their cytoplasm is rich with Nissl granules. The cells of parvocellular part are rounded with well defined nucleus (arrow head). (Gallocyanine stain ×1000).

Fig. 37 a: A photomicrograph of the cerebellum of twenty days old rat offspring of mother treated with cisplatin. It shows that the nuclei of some cells of fastigial nucleus have peripheral chromatin condensation (arrow head). The cytoplasm of many cells appears to be vacuolated (arrow).

Fig. 37 b: A photomicrograph of the cerebellum of twenty days old rat offspring of mother treated with cisplatin. The cells of magnocellular part of dentate nucleus are lightly stained (arrow). The cells of parvocellular part have darkly stained nucleus with vacuolated cytoplasm (arrow head). (Gallocyanine stain×1000).

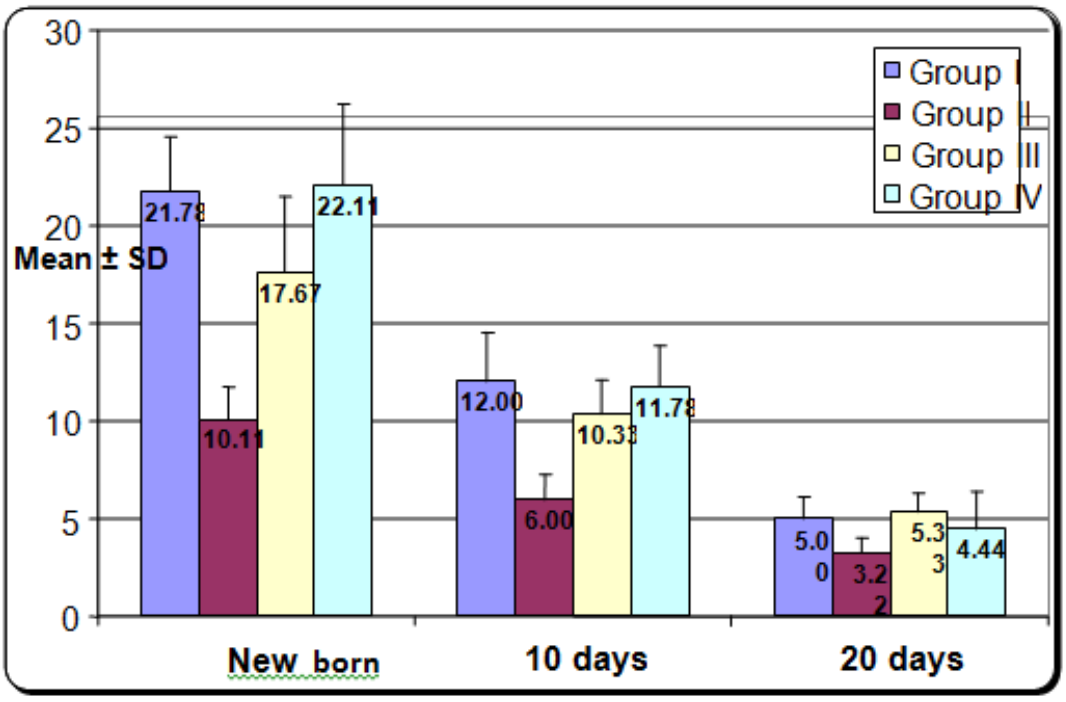
Fig. 38 a: A photomicrograph of the cerebellum of twenty days old rat offspring of mother treated with alpha lipoic acid and cisplatin. It shows that most of cells of fastigial nucleus nearly similar to those of the control. Few cells with darkly stained nuclei and vacuolated cytoplasm could be observed(arrow).

Fig. 38 b: A photomicrograph of the cerebellum of twenty days old rat offspring of mother treated with alpha lipoic acid and cisplatin. It shows that the cells of magnocellular part of dentate nucleus have rounded nuclei with prominent nucleolus (arrow). The cytoplasm is rich with Nissl granules. The cells of parvocellular part of dentate nucleus have rounded nuclei with uniformly distributed chromatin (arrow head). Note that it appears nearly similar to that of the control. (Gallocyanine stain×1000).

Morphometric results:

Table 1: The number of purkinje cells per an area 12360μ² in all studied groups at the different ages

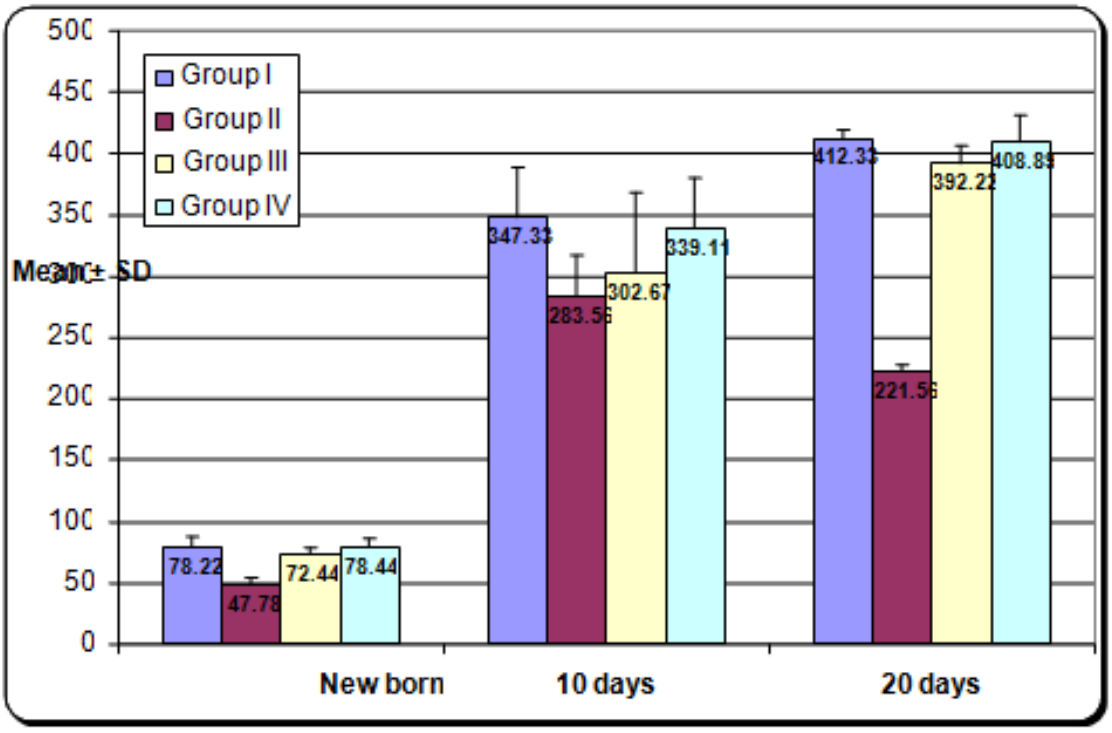
	Group I Mean ± SD	Group II Mean ± SD	Group III Mean ± SD	Group IV Mean ± SD
New born:				
Mean ± SD	21.78 ± 2.82	10.11 ± 1.62	22.11 ± 4.14	17.67 ± 3.81
Range	18.0-26.0	8.0-13.0	17.0-29.0	12.0-23.0
P-value[1]		0.000*	1.000	0.027*
P-value[2]			0.000*	0.001*
P-value[3]				0.057
10days:				
Mean ± SD	12.00 ± 2.55	6.00 ± 1.32	11.78 ± 2.11	10.33 ± 1.80
Range	8.0-15.0	4.0-8.0	8.0-14.0	8.0-14.0
P-value[1]		0.000*	0.654	0.165
P-value[2]			0.000*	0.000*
P-value[3]				0.141
20days:				
Mean ± SD	5.00 ± 1.12	3.22 ± .83	4.44 ± 1.94	5.33 ± 1.00
Range	4.0-7.0	2.0-4.0	2.0-8.0	4.0-7.0
P-value[1]		0.003*	0.344	0.462
P-value[2]			0.202	0.001*
P-value[3]				0.207



Histogram 1: It shows the relation between the number of cells in the purkinje layer in all the studied groups in different ages.

Table 2: The number of granule cells in the internal granular layer per an area 12360μ² at the different ages in all studied groups.

	Group I Mean ± SD	Group II Mean ± SD	Group III Mean ± SD	Group IV Mean ± SD
New born:				
Mean ± SD	78.22 ± 9.05	47.78 ± 6.44	78.44 ± 7.84	72.44 ± 7.13
Range	65.0-90.0	40.0-58.0	67.0-90.0	65.0-85.0
P-value[1]		0.000*	0.965	0.156
P-value[2]			0.000*	0.000*
P-value[3]				0.093
10days:				
Mean ± SD	347.33 ± 41.07	283.56 ± 33.99	339.11 ± 41.65	302.67 ± 65.90
Range	285.0-410.0	237.0-332.0	279.0-414.0	220.0-370.0
P-value[1]		0.005*	0.401	0.250
P-value[2]			0.012*	0.566
P-value[3]				0.508
20days:				
Mean ± SD	412.33 ± 7.60	221.56 ± 6.58	408.89 ± 22.62	392.22 ± 13.81
Range	400.0-420.0	215.0-230.0	365.0-437.0	370.0-414.0
P-value[1]		0.000*	0.894	0.003*
P-value[2]			0.000*	0.000*
P-value[3]				0.070



Histogram 2: It shows the relation between the number of granule cells in the internal granular layer in all the studied groups at the different ages

DISCUSSION

The cerebellum of rodents is a favorite object for the study of effects of exogenous factors on early processes of cell and tissue differentiation of the central nervous system[11].

In this study, the developing cerebellum of newborn rat of the control group was characterized by the presence of external granular layer. This layer was differentiated in to 2 zones which were premigratory zone and multiplying zone more superficially.These findings were in accordance with Schilling[12] who found that the external granular layer (EGL) is a temporary germinal matrix that gives rise to the granule cells. This germinal matrix was composed of two regions the proliferative zone, just beneath the pia matter, where the precursors of GCs (granule cells) are located, and the premigratory zone which characterized by the postmitotic granule cells that will migrate across the molecular layer to settle and constitute the internal granular layer (IGL).

In this work, in the new born rat of control group, two types of Purkinje cells could be seen. There was a high concentration of cells with darkly-staining lobulated polymorphous nuclei. Also, it was found that the purkinje cells arranged in multiple rows. This was in agreement with Altman[1] who reported that the Purkinje cells are the most numerous cellular elements of the cerebellar cortex of the newborn rat and they are distributed haphazardly about 6-12 cells deep between the thin molecular layer and the internal granular layer.

It was also observed that the layer that situated deep to the purkinje layer was the internal granular layer in which the cells were widely dispersed. This was in agreement with Altman[13] who stated that in the newborn and three-day old rats. The granule cells were extremely loosely packed in the transitional region between the Purkinje cell zone and the medullary layer, the future site of the granular layer.

At 10 days old rats of control group, the present result demonstrated that the external granular layer was still present with its 2 zones (multiplying zone and premigratory zone) with increase in its thickness with progress of development. This finding was in accordance with (Altman and Bayer[14]) who stated that if the rate of mitosis in the proliferative zone is much more than the rate of transformation, this resulted in an accumulation of cells in the premigratory zone. Hence the thickness of the external granular layer increased up to ten rows of cells between the eighth and ninth day of life.

In this study, the molecular layer at this age of the control group showed columns of migration of granule cells to reach its final destination in the internal granular layer. The cells of the internal granular layer were composed of rounded nuclei with peripheral chromatin condensation. In consistent with these results Hatten[15] found that the postmitotic cells of the external granular layer migrate to their final destination in the internal granule cell layer (IGL). The correct site of the cells is important for the final cytoarchitecture and for the pattern of synaptic connections in laminated brain regions such as the cerebellum[16]. The purkinje cells at this age were still arranged in several rows which was a sign of immaturity.

In this study, at the age of 20 days old rat there was only few cells can be observed in the external granular layer. In concomitant with this Cerri and collegues[17] stated that this layer was the matrix which generate the granule cells of the internal granular layer (IGL), after their migration in between Bergmann radial glial fibres.

Sudarov and Joyner[18] concluded that the Bregmann radial glia represented the mechanical power of cerebellar folia arrangement for the achievement of the normal cerebellum architecture.

In this study, the newly born rats whose mothers treated with cisplatin, the external granular layer showed increased in thickness as compared with control group. The increase in the thickness of the external granular layer was attributed to the delay of migration of the neurons of the granule cells to reach its final destination at the internal granular layer which is a sign of delayed growth.

In this present study, at 10 and 20 days old rat whose mother received cisplatin it was found that the cells of the premigratory zone had darkly stained nuclei. These results were in accordance with Avella and collegues[19] who found cell death in the proliferating external granular layer (EGL),

changes in granule cell migration from EGL to internal granular layer (IGL). These finding could be explained by Rzeski[20] who stated that the anticancer agent cisplatin has neurotoxic properties. Changes in the generation activity of the external granular layer (EGL) and in cell migration have been shown to occur after cisplatin administration[21]. Scherini and Bernocchi[22] found that the acute effects of cisplatin administration during postnatal development of the cerebellum are cell death in the external granular layer and alterations of granule cell migration.

The present ultrastructural study demonstrated the presence of degenerative changes of purkinje and granule cells. These results were in accordance with Pisu[21] who found degeneration of differentiating Purkinje cells after administration of cisplatin. It was also supported by Canta[23] who stated that neurotoxicity caused by cisplatin was due to the mitochondrial damage in the cells. Pisu[21] provide evidence that remodeling of the Purkinje cell dendrite and reorganization of the cerebellar cortex architecture occur, starting from a week after the cisplatin injection. The reorganization includes active neurogeneration of the (external granular layer) EGL. Rearrangement of Purkinje cell dendrite branches and the related synaptogenesis with inhibitory and excitatory fibers are visible[19].

In addition, Barrera[24] explained that chronic use of cisplatin increased the level of free oxygen radicals followed by reducing antioxidant production resulting in lipid membrane peroxidation and finally lead to tissue damage. Markers of neurotransmission molecules, such as glutamate and its ionotropic and metabotropic receptors, and GABA receptors in the purkinje cells were also affected by cisplatin treatment[25].

The purkinje cells at new born, 10 and 20 days old rats whose mother received cisplatin showed marked degenerative changes. The presynaptic terminal showed reduction of synaptic vesicles. These results could be explained by Ferguson[26] who stated that in the developing cerebellum neurotoxic agents destroy the cytoarchitecture and alter the pattern of connections established by fibers, neurite outgrowth and synaptic structuring. Platinum drugs appear to affect the nerve cell axons, myelin sheath, neuronal cell bodies, and the glial structures of the neural tissue[27].

In this study, there was statistically significant decrease in the number of purkinje and granule cells in 10 and 20 days old rat of cisplatin received group as compared with control group. These findings were in accordance with Avella[19] who found that after cisplatin administration there was alterations of the Purkinje cell dendrite growth. 20% of Purkinje cells degeneration has also been described[22].

These results could be explained by Pisu[28] who stated that in the postnatal development of rat cerebellum, the increased vulnerability of nerve cells to glutamate, through a mechanism that includes the inotropic and metabotropic receptors of this amino acid, provides evidence that the neurotoxicity of cisplatin has an excitotoxic component.

The present immunohistochemical study using anti glial fibrillary acidic protein revealed an increase in the glial fibers and the cells in all layers of the crebellar cortex in the group which treated with cisplatin only as compared with control group.

These results were in concomitant with Imosemi[29] who found increased expression of GFAP was seen in the cisplatin-treated rats compared with the control rats, which was indicative of astrocytic response to cisplatin injury (increased astrocyte activation), resulting in astrogliosis. The mechanism involved in this over expression of GFAP was unclear, however, cisplatin has been reported to affect neuronal cell body, glial structures of the neurons[27], interferes with DNA replication and function of the neurons[30], thus increasing GFAP expression. Over expressed glial cells had been shown to secrete a number of factors with pro-inflammatory and neurotoxic properties including cytokines, and free radicals such as nitric oxide (NO)[31].

This study showed that neurons of the fastigial nucleus and the magnocellular part and parvocellular part of dentate nuclei were lightly stained at 10 and 20 days old rat of cisplatin received group which indicate marked loss of Nissl granules. These results were due to neurotoxic activity of cisplatin. Almutairi[32] stated that oxidative damage was one of the important mechanisms suggested for the neurotoxicity induced by cisplatin. The increasing level of oxidative stress changed the cell structure and

disturbed the cell functions as well as decreased the antioxidant mechanism causing DNA damage in biological systems[33].

In this study, the administration of both alpha lipoic acid and cisplatin to pregnant and lactation females revealed that the structure of purkinje and granule cells were nearly similar of that of control. Only few vacuoles could be observed in the cytoplasm of the cells.

There was reduction in the immunoreactive cells and fibers in the cerebellar cortex as compared with cisplatin received group. These finding could be explained by Bhadri[34] who stated that alpha lipoic acid has a protective effect on the neurons. Alpha lipoic acid was a powerful antioxidant capable of prevention or treatment of many diseases causes oxidative stress, such as diabetes, chronic liver diseases, and neurodegenerative processes[35]. ALA was able to improve the levels of endogenous antioxidants and decreased biochemical abnormalities such as the lipid peroxidation[36].

There was statistically insignificant difference in the number of purkinje cells and granule cells of ten and twenty days old rats whose mothers received cisplatin and alpha lipoic acid as compared with control group. This was in accordance with Condo[37] who found that alpha lipoic acid induced the expression of frataxin, a mitochondrial protein with anti-oxidant and anti-apoptotic properties suggesting a possible role in regulating degenerative and protective pathways in neurons.

It was also supported by Melli[4] who showed that alpha lipoic acid produces neuroprotective effects against chemotherapy - induced neurotoxicity in vitro, preventing axonal damage and mitochondrial dysfunction in sensory axons[38]. Tuncer[7] concluded that both alpha lipoic acid and melatonin have the capacity to prevent cisplatin induced neurotoxicity.

The examination of cerebellar nuclei of the developmental group whose mothers received both alpha lipoic acid and cisplatin showed that the cells had regular outline with well defined nuclei. This finding was attributed to the antioxidant activity of alpha lipoic acid. Our finding could be explained by Mantovani[5] who found that Alpha-lipoic acid (aLA) was important for cell energy metabolism, and it was a cofactor at entry to Krebs cycle, displayed anti-oxidant effects by increasing the glutathione peroxidase activity and reducing oxidative stress, regulating calcium homeostasis[39] and regulating the activity of the transcription factor NF-kB[38].

In conclusion, the cisplatin has neurodegenerative effect on the cerebellum of both developmental and aged groups. The alpha lipoic acid has antioxidant effect against the degenerating effect of cisplatin when it is used in combination with cisplatin.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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دراسة عن تاثير اعطاء عقار السيسبلاتين علي المخيخ في الفئران البيضاء النامية والدور الوقائي المحتمل للالفاليبويك اسيد

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ملخص البحث

يلعب المخيخ دور مهم في وظائف الدماغ العليا. يستخدم السيسبلاتين في علاج الاورام الخبيثة. يؤدي العلاج بالسيسبلاتين الي عدة مشاكل وظيفية. يوجد الالفا ليبويك أسيد في ميتوكوندريا الخلايا حيث مكان توليد الطاقة في الخلية. تمت هذه الدراسة الجارية للتعرف علي علي نمو المخيخ بعد الولادة ومعرفة تاثير عقار السيسبلاتين واستخدامه بالتزامن مع الالفا ليبويك اسيد علي المخيخ في الفئران النامية من خلال فحص بالميكرسكوب الضوئي ودراسه تركيبية دقيقة وهستوكيميائية مناعيه ودراسة مورفوميتريه. لقد استخدم عدد 40 انثي فار ابيض حامل. قسمت هذه الفئران الى اربع مجموعات :مجموعه ضابطه ومجموعه اعطيت عقار السيسبلاتين ومجموعه أعطيت الالفا ليبويك اسيد ومجموعه اعطيت كلا من السيسبلاتين والالفا ليبويك اسيد. الاعمار التي تم دراستها هم حديث الولادة وعمر 10 ايام وعمر 20 يوم. ولقد اظهرت الدراسة ان السيسبلاتين له تاثير مدمر للخلايا العصبيه في طبقة بيركنجي والطبقه المحببة في قشرة المخيخ وفي منطقة الانويه العميقه في المخيخ. أظهرت الدراسة المورفومترية نقصا ذو دلالة احصائيه في خلايا بيركنجي والخلايا المحببه في المجموعة التي اعطيت السيسبلاتين عند مقارنتها بالمجموعه الضابطه. ولقد اظهر الالفا ليبويك اسيد تحسنا في معظم التغيرات التي حدثت بسبب السيسبلاتين.