Original Article

Effect of Different Doses of Pregabalin on the Postnatal Development of the Cerebellar Cortex in Albino Rats

Salwa M. Ouies[2], Zahraa M. Ismael[2], and Moustafa A. Abdellah[1]

[1]Department of Obstet/ Gynecol, [2]Department of Human Anatomy and Embryology, Faculty of Medicine, Sohag University, Egypt

ABSTRACT

Background: Pregabalin is an oral medication that is used to treat neuropathic pain, generalized anxiety disorder and epilepsy; as a therapy for partial seizures. Cerebellar development is not complete during gestation only; but continues after birth to maturation of the cerebellum (postnatal development).

Aim of the Work: To study the possible effect of Pregabalin on the postnatal development of the cerebellar cortex of albino rat.

Material and Methods: 60 pregnant albino rats were allocated into three groups; Group A: Included 20offspring of 20 control mothers, Group B: Included 20 offspring of 20 mothers treated orally with pregabalin at a dose of 150mg/kg, and Group C:included 25 offspring of 20 mothers treated orally with pregabalin at a dose of 600mg/kg. In the three groups rats were sacrificed at postnatal (age 1, 2, 3 weeks), in addition to adult age (2.5 months).The skulls were opened and the cerebella were removed and processed for light and transmission electron microscope.

Results: Pregabalin administration during pregnancy and lactation caused its marked effect during early postnatal life and this effect extended till the adult stage; in the form of increase in the external granular layer (mainly in the 2nd and 3rd weeks); less thickness of the molecular layer in all treated groups; shrunken and abnormal shaped Purkinje cells which appeared with ill-defined cell membranes and destructed nuclei; mainly at higher dose. There were also delayed differentiation of the internal granular layer and granule cells appeared with destructed cytoplasmic membrane and organelles which appeared prominent at higher doses.

Conclusion: Pre and postnatal administration of pregabalin in both low and high doses caused loss of cellular components, distortions of cerebellar cortical cells in a dose dependent manner.

Received: 31 August 2020, **Accepted:** 23 October 2020

Key Words: Cerebellum, postnatal rats, pregabalin, purkinje cells.

Corresponding Author: Salwa Mohammed Ouies, MD, Department of Human Anatomy and Embryology, Faculty of Medicine, Sohag University, Egypt, **Tel.**: +20 1002073124, **E-mail:** salwaouies@yahoo.com

ISSN: 1110-0559, Vol. 44, No.3

INTRODUCTION

The cerebellum (the largest part of the hindbrain) lies in the posterior cranial fossa, posterior to the pons, medulla and fourth ventricle. It is connected to the brainstem by three paired cerebellar peduncles. The cerebellar surface has a numerous curved transverse fissures giving its laminated appearance and separating between its folia[1]. The cerebellar cortex consists of three layers of cells outer molecular, middle Purkinje, inner granular, and five types of neurons; basket, stellate, Purkinje, Golgi type II and granule neurons[2].

The cerebellar cortex in early postnatal stages of development is completely different from that of adult type, mainly due to the presence of the external granular layer at early stages of cortical development[3].

The cerebellum is very sensitive to the abnormal changes during the embryological development in its histological structure; this may be due to the maternal exposure to chronic or acute diseases or exposure to certain chemicals (drugs or toxicants) during early term of pregnancy[4].

Pregabalin is the latest compound that joins the list of approved new Antepileptic drugs, AEDs. The European Commission granted Pfizer Company the approval for pregabalin in July 2004 for the treatment of variable types of peripheral neuropathic pain and in the same year, it was approved as adjunctive therapy for epilepsy by the American Food and Drug Administration (FDA)[5].

It has been shown that pregabalin can cross easily the placenta and the blood-brain barrier in rats, mice, and monkeys. However, data on pregabalin use during human pregnancy are limited. A significant increase in the risk of major congenital disabilities after exposure to pregabalin in the first trimester was reported by a recent prospective study[6]. Therefore, pregabalin is an important drug that can influence the development of the nervous system after birth[7].

AIM OF THE WORK

To study the possible effect of two doses of Pregabalin on the postnatal development of the cerebellar cortex of albino rat by histological and transmission electron microscope examinations.

 DOI: 10.21608/ejh.2020.36819.1350

MATERIAL AND METHODS

Materials: Pregabalin (Lyrica) capsules of 150 mg from Pfizer-Egypt Company.

Animals

90 albino rats; 60 adult females and 30 adult males, weighing 200-250g, were utilized for mating. These rats were obtained from the Animal House of Assiut Faculty of Medicine. They were housed in the Animal House, Sohag University, Egypt. All rats were allowed free access for rodent chow diet and water. The experiment was performed according to the "Guide for the Care and Use of Laboratory Animals" (Institutes of Laboratory Animal Research)[8] and in accordance with the guidelines of the Animal Ethics and approved by Research Ethics Committee considering care and use of laboratory animals at Sohag University.

Experimental design

After a 7-day acclimatization period, adult females were housed with adult males at ratio of 2:1 in each cage respectively. Then, vaginal smears were taken in next day to detect occurrence of sperms. Pregnancy was calculated from the 1st day of the positive vaginal smear. Pregnant female rats were equally divided into three groups as follow:

Group A (control group): contained 20 pregnant rats which did not received any treatment.

Group B: contained 20 pregnant rats which were given pregabalin at dose of 150mg/kg by gastric intubation daily[9].

Group C: contained 20 pregnant rats which were given pregabalin at dose of 600mg/kg by gastric intubation daily[9].

Drug, dosage and administration

For group B a capsule of 150 mg dissolved in 4ml distilled water to give 37.5mg/ml solution, for each 250 gm rat 1ml of the prepared solution given, and for group C a capsule 150 mg dissolved in 1ml, each 250 gm rat given 1ml.The pregnant females were treated from the 1st day of the positive vaginal smear till birth and during whole period of lactation (up to postnatal day 21).

Allocation of groups

At birth, each mother was housed with its young rats in a large cage in a ventilated room at a constant temperature (25°c.) with a12:12 h light/ dark cycle. Each group contained first generation of male young rats with their mothers. Then, the pups were divided into three subgroups (5 male young rats for each) according to the postnatal day of scarification as follow: Subgroups AI, BI and CI: sacrificed after 7 days postnatally (PD7). Subgroups AII, BII and CII: sacrificed after 14 days postnatally (PD14). Subgroups AIII, BIII and CIII: sacrificed after 21 days postnatally (PD21) in addition to Subgroups A IV, B IV and C IV: sacrificed at adult age (2.5 months).

Methods

The animals were sacrificed 24 h after the last dose; the cerebellum was dissected, formalin fixed and processed for histological examination by H and E and examined by an Olympus light microscope[10].

Small pieces were also cut, fixed in 2.5% glutaraldehyde and processed for transmission electron microscopic examination, semithin sections were stained with toluidine blue, sections were then examined by an Olympus light microscope to choose the selected areas. Ultrathin sections, 50–80 nm, were cut from selected areas and examined in the transmission electron microscope unit, Assuit University[11].

Morphometric study and statistical analysis

Estimation of the diameters of Purkinje cells of control and pregabalin treated rats were done for 3wks and adult groups: The longest axis of the Purkinje cells (average of greatest and least dimensions for each cell) was measured as the major diameter[12] (At magnification 1000) toluidine blue stained sections.

The previous parameter was measured using an image analysis system (Digimizer; Version 3.7. 2005-2010 MedCalc Software).

Variables were represented by mean ± Sd (Mean ± standard deviation of mean). The SSPS program version 16 was used to analyze the differences among all groups in all the data parameters by one-way analysis of variance and a post-hoc test was used to find the statistical difference between the groups when ANOVA was statistically significant (*P value* ≤0.05)[13].

RESULTS

The pregnant rats treated with pregabalin showed no external signs of toxicity, no mortality cases were recorded, and all the treated rats were survived to the end of study

A-Light microscopic examination

1-Cerebellar sections of PD7 albino rats (subgroups AI, BI and CI)

In control albino rat (AI) the normal architecture of cerebellar cortex at this age appeared; the external granule layer appeared as a thick superficial layer of small oval cells with small rounded deeply stained nuclei; the molecular layer started to appear as clear thin area on top of Purkinje cell layer; the Purkinje cell layer showed arrangement of large faintly stained cells arranged in one or two layers on top of internal granular layer, these cells acquired somewhat the adult appearance with oval shape and rounded vesicular nuclei; the internal granular layer was well developed, the cells appeared small in size, sparsely arranged with small deeply stained nuclei (Figure 1).

In group BI: The external granular layer showed a thick layer similar to the control group; the molecular layer appeared relatively not well-differentiated from Purkinje

cell layer; the Purkinje cell layer appeared not well cleared from the underlying layer; the internal granular layer showed aggregation of darkly stained cells as appeared in the control group (Figure 2).

In group CI: The external granular layer showed marked decrease in the thickness compared to the control group with areas of discontinuations, fissures and hemorrhage; the molecular layer relatively exhibited more cellular population than that of the control rats; the Purkinje cell layer appeared as dark line on top of granule cell layer, its cells appeared not well differentiated from surrounding granule cells; the internal granular layer showed less aggregations of cells than control, cells appeared darkly stained with multiple spaces in-between them (Figure 3).

2- Cerebellar sections of PD14 albino rats (subgroups AII, BII and CII)

At PD14, the cerebellum of control albino rat (AII) showed a very thin superficial external granule layer l formed of small oval cells with small rounded deeply stained nuclei; the molecular layer appeared very clear and well differentiated wide layer on top of the Purkinje cell layer contained many rounded and oblong cells. The molecular layer appeared to reach its maximum cellularity at this age; the Purkinje cell layer showed well differentiated cells arranged in one or two rows, these cells acquired somewhat the adult appearance with oval shape and rounded vesicular nuclei and prominent nucleoli; The internal granular layer was well developed; the cells appeared small in size, packed together with small deeply stained nuclei and rim of acidophilic cytoplasm (Figure 4).

At BII subgroup: The external granular layer showed some increase in the thickness compared to the control group with deeply stained cells.

The molecular layer relatively exhibited more cellular population than that of the control rats; the Purkinje cell layers appeared formed of multiple rows of smaller sized cells with loss of their oval characteristic shape; the internal granular layer showed aggregation of cells in the form of follicles or rosette shape (Figure 5).

At CII subgroup: The external granular layer showed marked increase in thickness compared to the control group; The molecular layer relatively exhibited more cellular population than that of the control rats , cells appeared migrating and still in continuation with the external granular layer; the Purkinje cell layer showed marked decrease in thickness and cellular destruction than control group, the cells became smaller in size with a lot of degenerated and pyknotic cells; The internal granular layer showed aggregations of cells in the form of follicles or rosette shape (Figure 6).

3- Cerebellar sections of PD21 albino rats (subgroups AIII, BIII and CIII)

At PD21 of control albino rats (AIII) the cerebellar cortex of the 3weeks rats was formed of a remnant of external granular layer, molecular layer, Purkinje cell layer & inner granular layer.

The molecular layer: It appeared as a thick layer extends deeply towards the Purkinje cell layer, it contained scattered nuclei. The nuclei of this layer were of different sizes & shapes; the Purkinje cell layer appeared as a narrow zone constituting a single row of nuclei parallel to the surface of the folia, it present just superficial to the granular cell layer. The Purkinje cells appeared large, faintly stained having an oval shape. The nuclei appeared relatively large, vesicular and nearly filling the cell bodies. The granular cell layer: The granular layer was clearly defined and lies deep to the Purkinje cell layer of the cerebellum. Granule cells appeared with their small rounded cell bodies (Figures 7,8).

BIII subgroup: the External granular layer: remnants of cells were presented.

The Molecular layer: with more scattered cell in comparison with control; at Purkinje cell layer showed atypical cells with ill define cytoplasm and ill-defined nuclei. Some Purkinje were shrunken deeply stained with pyknotic nuclei; In the Granular cell layer; the cells appeared clumped into groups, some of them appeared as control group other were pyknotic (Figures 9,10).

CIII subgroup showed the External granular layer as a thin prominent rim external to the molecular layer

Molecular layer: Superficial to Purkinje cell layer show scattered few cells with small shrunken cells; Purkinje cells: appeared atypical shrunken with ill-defined cytoplasm and other cells appeared pyknotic; Granular cell layer: Showed small sized darkly stained cells, vacuoles appeared in between Purkinje and Granule cells (Figures 11,12).

4- Cerebellar sections of adult albino rats (subgroups AIV, BIV and CIV)

The cerebellar cortex of the adult Control adult rats (AIV) showed

The molecular layer: appeared as a thick layer extends deeply towards the Purkinje cell layer, it contained scattered nuclei. The nuclei of this layer were of different sizes & shapes. Some of them were rounded, others were elongated and satellites in shape.

The Purkinje cell layer: appeared as a narrow zone constituting a single row of nuclei parallel to the surface of the folia, it present just superficial to the granular cell layer and in the deepest part of the molecular layer.

The Purkinje cells appeared large, having a pear shape. The nuclei appeared relatively large, and nearly filling the cell bodies. They were vesicular and contained few chromatin granules mostly peripheral in position. Most nuclei contained one or more dark well distinct nucleoli.

The granular cell layer: was clearly defined, crowded with cells and lies deep to the Purkinje cell layer of the cerebellum. Granule cells clumped in groups appeared

with their small rounded cell bodies. The nuclei filled the cell and had fine chromatin granules and some of them contained well developed nucleoli (Figures 13,14).

BIV subgroup: Molecular layer appeared with less scattered cells and empty spaces in compare with control group. Purkinje cells appeared atypical and shrunken, darkly stained with small pyknotic nucleoli, multiple vacuoles appeared around some of them.

The Granule cells appeared clumped into group, some of them appeared as control group other were pyknotic (Figures 15,16).

CIV subgroup: Molecular layer showed scattered few cells with small shrunken cells. Purkinje cells appeared shrunken, atypical with ill-defined cytoplasm and other cells appeared pyknotic. Granular cells appeared as groups of small sized darkly stained cells (Figures 17,18).

B- Electron microscope study

1-PD21 albino rats (subgroups AIII, BIII and CIII)

Ultrastructural examination of 3 weeks control rats (AIII subgroup) showed:

The Purkinje cell appeared as a large cell with euchromatic nucleus and prominent nucleolus surrounded with well- defined cytoplasmic membrane and organelles; mitochondria and intact rough endoplasmic reticulum was observed (Figure 19).

The granular cells appeared more or less equal in size, each cell appeared with large euchromatic nucleus and thin layer of cytoplasm having few organelles as mitochondria and strands of rough endoplasmic reticulum (Figure 20).

BIII subgroup

Purkinje cell appeared having irregular outlines, it had electron dense nucleus with prominent nucleolus, the cytoplasm contained scattered mitochondria with destructed cristae and dilated rough endoplasmic reticulum was observed (Figure 21).

Granular cell appeared with large nucleus had regular nuclear envelope, dens chromatin and surrounded by well-defined cytoplasmic membrane (Figure 22).

CIII subgroup

Purkinje cells: appeared destructed compared to the controls with ill-defined irregular cell membrane, destructed nucleus with ill-defined membrane and dilated rough endoplasmic reticulum (Figure 23).

Granular cells: appeared with destructed cell membrane and organelles, large irregular nuclei with discontinued nuclear envelop, chromatin crowded peripherally with marked cytoplasmic and nuclear vacuoles. (Figure 24)

2-Adult albino rats (subgroups AIV, BIV and CIV)

Ultrastructural examination of adult control rats (AIV subgroup) showed

The Purkinje cell: appeared as a large cell with well-defined membrane and cytoplasm with intact cytoplasmic organelles, mitochondria appeared with intact crystals and intact rough endoplasmic reticulum was observed, the nucleus was large, euochromatic with prominent nucleolus (Figure 25).

The granular cell: appeared more or less equal in size with large nucleus containing clumps of chromatin and thin layer of cytoplasm having few organelles as mitochondria and strands of rough endoplasmic reticulum (Figure 26).

At BIV subgroup

Purkinje cell: appeared deeply stained with irregular cell membrane, electron dense nucleus with irregular ill-defined nuclear membrane condensed chromatin and prominent nucleolus, the cytoplasm contain scattered mitochondria with destructed cristae and dilated rough endoplasmic reticulum (Figure 27).

Granular cell: appeared with large nucleus, regular nuclear envelope, dens chromatin and surrounded by ill-defined cytoplasmic membrane with multiple vacuoles in-between cells (Figure 28).

CIV subgroup

Purkinje cells: appeared markedly destructed with ill-defined cell membrane, destructed nucleus with irregular ill-defined nuclear envelop, smaller sized extruded nucleolus and destructed rough endoplasmic reticulum (Figure 29).

Granular cell layer: appeared with destructed cytoplasmic membrane and organelles, large nuclei with discontinued nuclear envelope, chromatin crowded peripheral with marked cytoplasmic and nuclear vacuoles (Figure 30).

Morphometric studies

The Purkinje cell diameter

A-3WEEKS RATS

The mean value of the Purkinje cell diameter in the treated group II was (207.9 um) which was very highly significant decreased compared to the control group (301.7 um) ($P \leq 0.000$) (Table1, Histogram 1).

The mean value of the Purkinje cell diameter in the treated group III was (204.3um) which was very highly significant decreased compared to the control group ($P \leq 0.000$). (Table 1, Histogram 1).

B- 2 MONTHS RATS

The mean value the of the Purkinje cell diameter in the treated group II was (168.06 um) which was very highly significant decrease compared to the control group (271 um) ($P \leq 0.000$) (Table1, Histogram1).

The mean value of the Purkinje cell diameter in the treated group III was (137.52um) which is very highly

significant decreased compared to the control group ($P \leq 0.000$) (Table 1, Histogram 1).

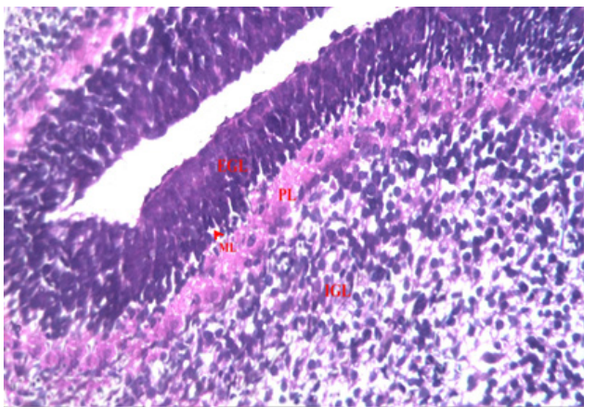


Fig. 1: A Photomicrograph of a sagittal section of 1week control cerebellum showing thick external granule layer (EGL), molecular layer (ML) start to appear as clear area (arrow head), Purkinje layer (PL) showed arrangement of large faintly stained cells, and internal Granule layer (IGL) crowded with deeply stained cells. (H&E X400)

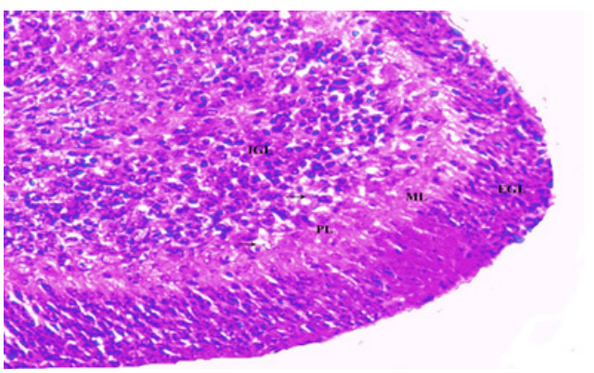


Fig. 2: A Photomicrograph of sagittal section of 1wk treated cerebellum group (II): showing thick external granule layer (EGL) which shows dark apoptotic nuclei in all layers (p), molecular layer (ML) deep to external granular layer not well differentiated from Purkinje layer (irregular arrow), Purkinje layer (PL)appear not clear from the underlying layer, internal Granule layer (IGL)appear crowded with cells. (H&E X400)

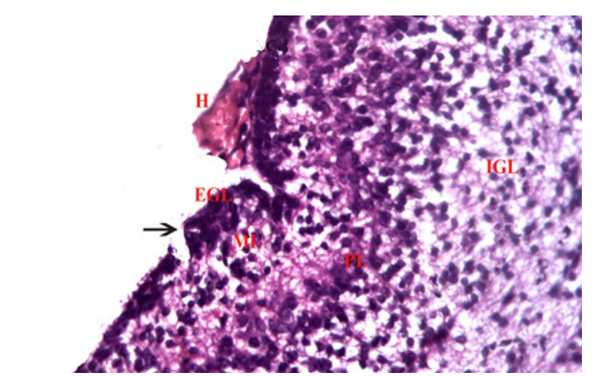


Fig. 3: A Photomicrograph of sagittal section of 1wk treated cerebellum group (III):showing the external granule layer (EGL) has marked decrease in its thickness than control with some areas of hemorrhage (H) and fissures (thin arrows), molecular layer (ML) appear as loose cellular filled area and Purkinje layer (PL) not well differentiated from internal Granule layer (IGL). (H&E X400)

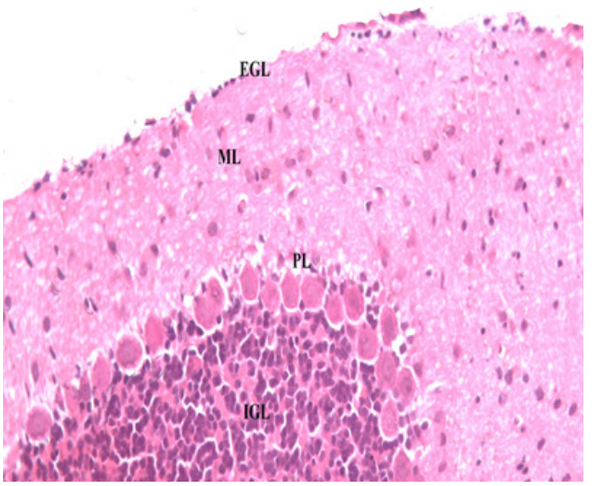


Fig. 4: A Photomicrograph of sagittal section of 2weeks control cerebellum showing different layers; thin superficial external granule layer (EGL), wide molecular layer (ML), well defined Purkinje layer (PL) with well-defined cells, and internal granule layer (IGL)crowded with granule cells arranged in groups.(H&E X400)

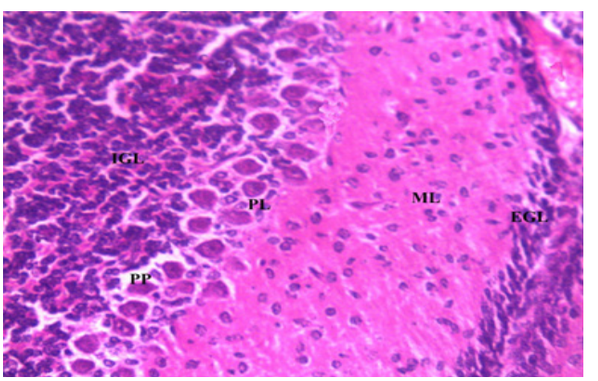


Fig. 5: A Photomicrograph of sagittal section of 2weeks treated group (II) cerebellum showing prominent external granule layer (EGL) slightly thicker than control , wide molecular layer (ML) more cellular than control, Purkinje layer (PL) formed of 1-2 rows of darkly stained cells with some pyknotic cells (PP), crowded internal granule layer (IGL) close to controls . (H&E X400)

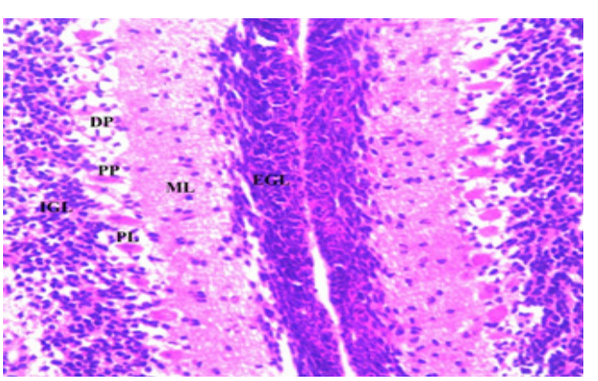


Fig. 6: A Photomicrograph of sagittal section of 2weeks treated group (III) cerebellum showing different layers: external granule layer (EGL)is markedly thicker than control, thin molecular layer (ML), Purkinje layer (PL) formed of deeply abnormal shapes cells with some pyknotic cells (PP) and degenerated cells (DP), internal granule layer (IGL) appear crowded with deeply stained cells. (H&E X400)

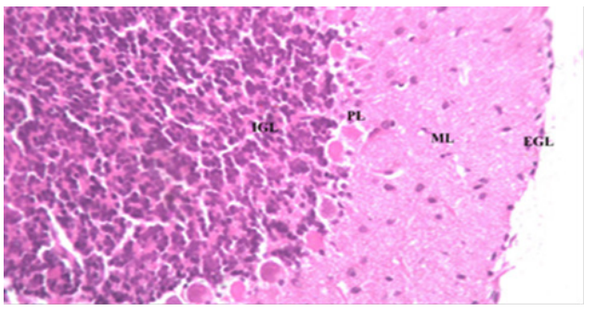


Fig. 7: A Photomicrograph of a sagittal section of 3weeks control cerebellum showing very thin external granule layer (EGL), wide molecular layer with scattered cells (ML),Purkinje layer (PL) formed of single row of cells and internal granule layer (IGL) crowded with cells arranged into groups. H&E X 400.

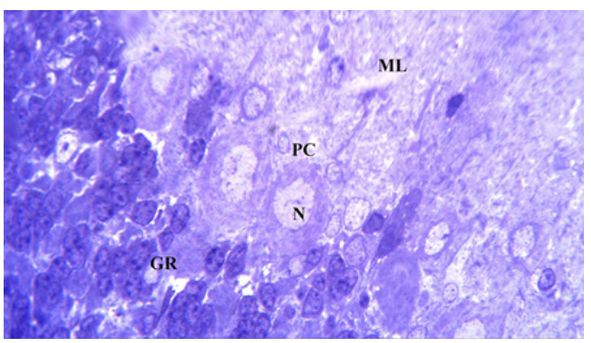


Fig. 8: A photomicrograph of a semi thin section of the cerebellar cortex of 3weaks control group showing molecular layer (ML) with scattered cells, Purkinje cells (PC) has a large oval shaped cell body with faintly stained large vesicular nucleus (N) and thin rim of cytoplasm, granular cells (GR) appeared crowded together with deeply stained rounded to oval nuclei. Toluidine blue × 1000

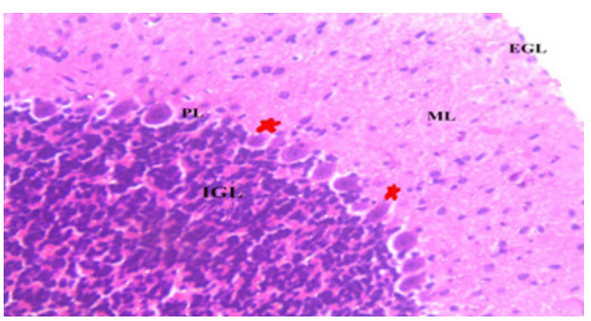


Fig. 9: Photomicrograph of sagittal section of 3weeks treated cerebellum group (II): showing a thin rim of external granule layer (EGL), wide molecular layer (ML) with more cellular contents, in Purkinje layer (PL) some cells are atypical and some are pyknotic (stars), crowded internal granule layer (IGL) with darkly stained cells. H&E X 400

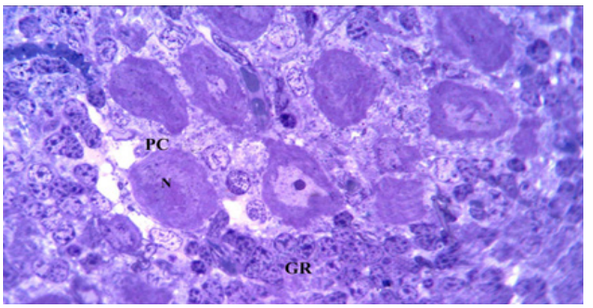


Fig. 10: A photomicrograph of a semi-thin section of 3weeks treated cerebellar cortex of group(II) showing Purkinje cells (PC) appear atypical and deeply stained with pyknotic nuclei (N), granular cells (GR) appear crowded with deeply stained rounded to oval cells. Toluidine blue × 1000

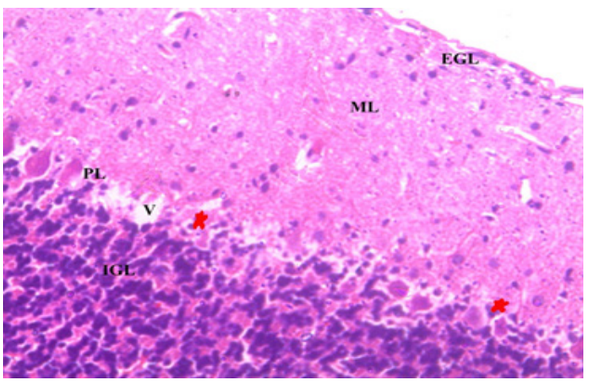


Fig. 11: A Photomicrograph of sagittal section of 3weeks treated cerebellum group (III): showing thin rim of external granule layer (EGL), wide molecular layer (ML) with some vacuoles (v), Purkinje layer (PL) show multiple vacuoles and are atypical and some are pyknotic (star), with crowded darkly stained cells in the internal granule layer (IGL). H&E X 400

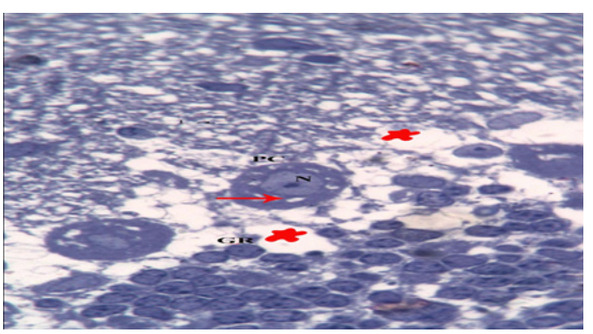


Fig. 12: A photomicrograph of a semi thin section of 3weaks treated cerebellar cortex of group (III) showing Purkinje cells (PC) with irregular shape, shrunken and deeply stained cells with pyknotic nuclei (N) and vacuolated cytoplasm(arrow), granular cells (GR) with crowded cells, vaculations appear in-between these cells(stars).Toluidine blue × 1000

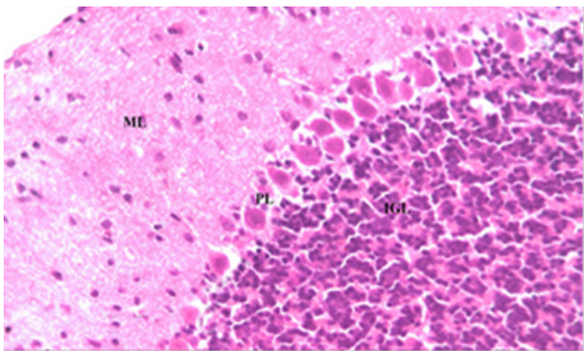


Fig. 13: Photomicrograph of sagittal section of adult control cerebellum showing well differentiated cortical layers; clear molecular layer (ML) with scanty scattered cells, Purkinje layer (PL) differentiated from other layers and internal granule layer crowded with cells arranged into groups (IGL). H&E X400

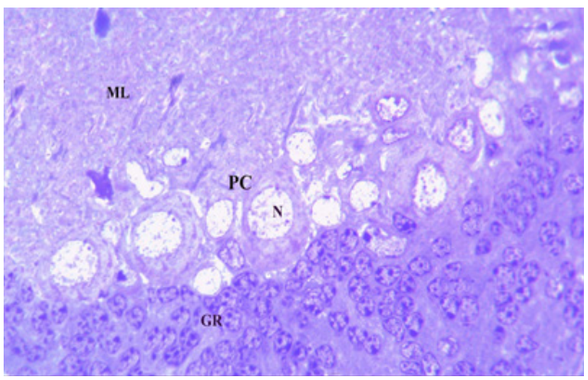


Fig. 14: A photomicrograph of a semi thin section of the cerebellar cortex of adult control group showing molecular layer (ML), Purkinje cells (PC) appear with large pear shaped cell bodies and faintly stained large vesicular nucleoli (N) and thin rim of cytoplasm, Granular cells (GR) appear oval to rounded cells with large oval nuclei. Toluidine blue × 1000

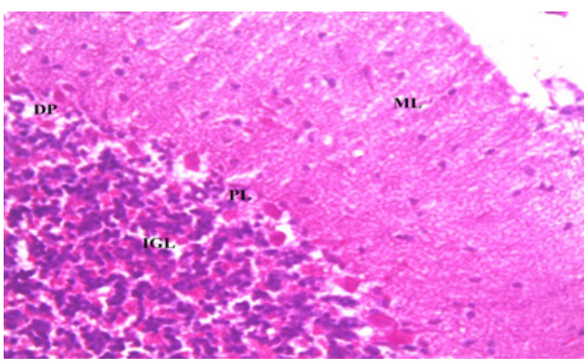


Fig. 15: Photomicrograph of sagittal section of adult treated cerebellum group (II) showing less scattered cells in the molecular layer (ML), Purkinje cell layer (PL) show multiple atypical deeply stained Purkinje cells (DP) and internal granule layer (IGL) appear crowded with darkly stained cells. H&E X400

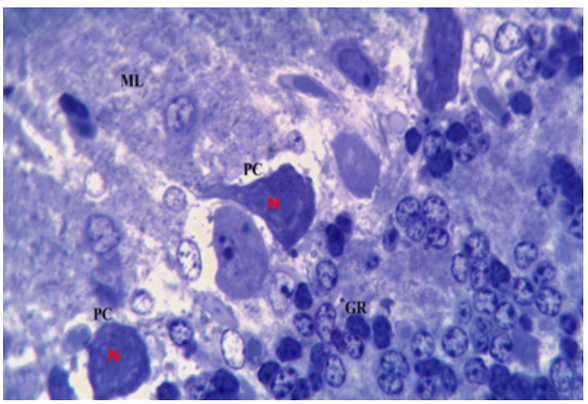


Fig. 16: A photomicrograph of a semi thin section of the cerebellar cortex of adult treated cerebellum group(II) showing molecular layer (ML), Purkinje cells (PC) appear shrunken, darkly stained with small pyknotic nuclei(N) and thin rim of cytoplasm, some granular cells (GR) appear pyknotic. Toluidine blue × 1000

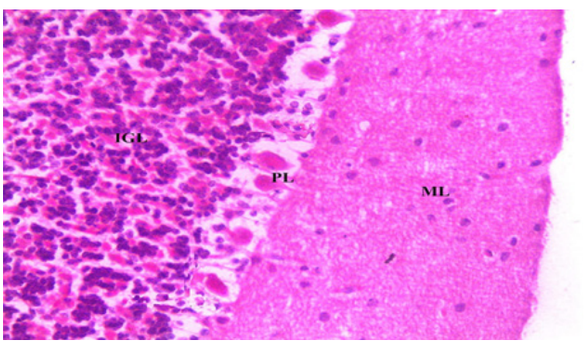


Fig. 17: Photomicrograph of sagittal section of adult treated cerebellum group (III) showing less scattered cells in molecular layer (ML),Purkinje cell layer show atypical pyknotic cells, internal granule layer (IGL) appear less crowded with cells than control group. H&E X400

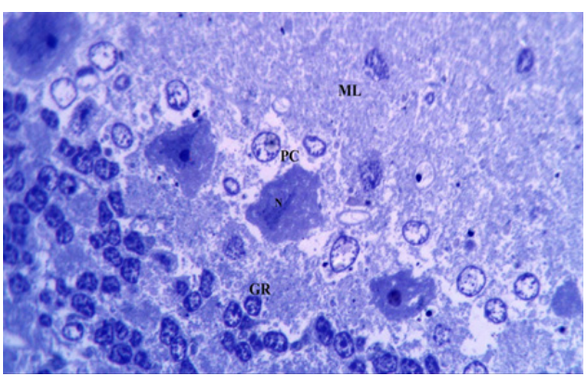


Fig. 18: A photomicrograph of a semi thin section of the cerebellar cortex of adult group (III) showing molecular layer (ML) with less scattered cell population than control. Purkinje cells (PC) are markedly shrunken darkly stained with pyknotic nucleoli (N), granular cells (GR) showing small sized darkly stained cells. Toluidine blue × 1000

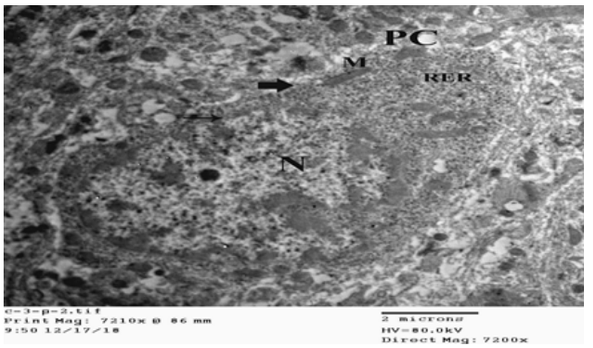


Fig. 19: An electron micrograph of the Purkinje cell of 3wks control rat cerebellum showing its characteristic oval shaped large cell (PC) with well-defined cell membrane (thick arrow), nucleus (N) appear with well-defined nuclear membrane (thin arrow) and prominent nucleolus, the cytoplasm appear with well-defined organelles; the mitochondria (M) are oval in shape and Rough endoplasmic reticulum (RER)are intact. (x7210)

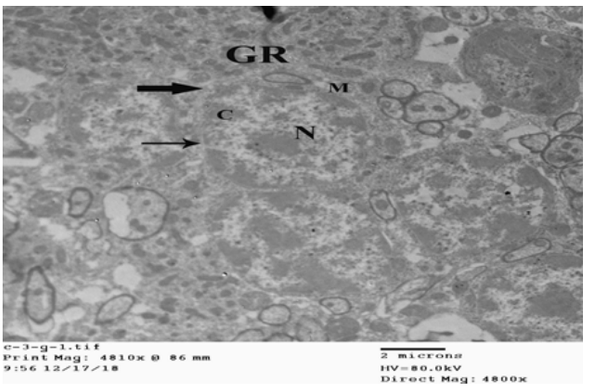


Fig. 20: An electron micrograph of 3wks control cerebellum showing a group of granular cells (GR) with large size euchromatic nucleus (N) with electron dense chromatin (C) which show well defined nuclear membrane (thin arrow) surrounded by thin rim of cytoplasm with well- defined cell membrane (thick arrow) and multiple mitochondria(M) (X 4810)

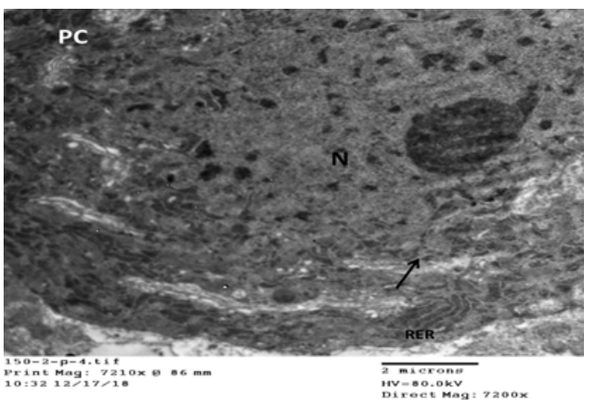


Fig. 21: An electron micrograph of group (II) of 3wks treated cerebellum showing Purkinje cell (PC) with electron dense nucleus (N) with prominent nucleolus and ill- defined nuclear envelop (arrow), cytoplasm show also dilated rough endoplasmic reticulum (RER). (X7210)

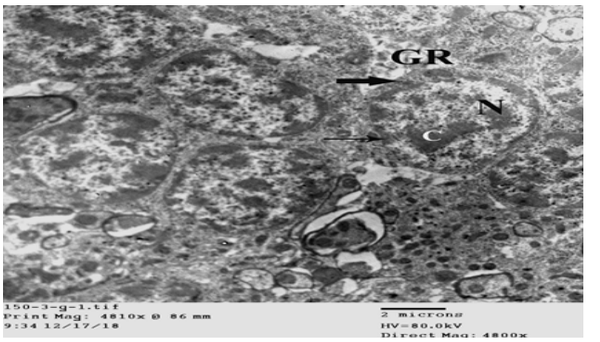


Fig. 22: An electron micrograph of the cerebellar cortex of (group II) 3wks treated cerebellum showing group of granular cells (GR) with large nucleus (N) surrounded by regular nuclear envelope (thin arrow), more condensed Chromatin (C), thin rim of cytoplasm surrounded by well-defined cell membrane (thick arrow).(x4810)

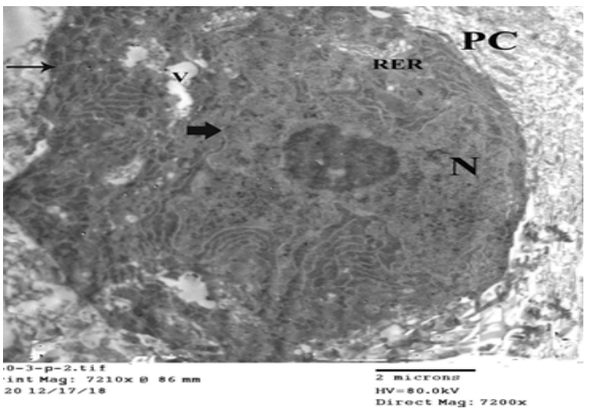


Fig. 23: An electron micrograph of the cerebellar cortex group (III) 3wks treated cerebellum showing atypical Purkinje cell (PC) with irregular cell membrane (Thin arrow), condensed chromatin nucleus (N) with ill-defined nuclear envelop (thick arrow), dilated rough endoplasmic reticulum (RER). Vacuoles (V) appear within the cytoplasm. (X7210)

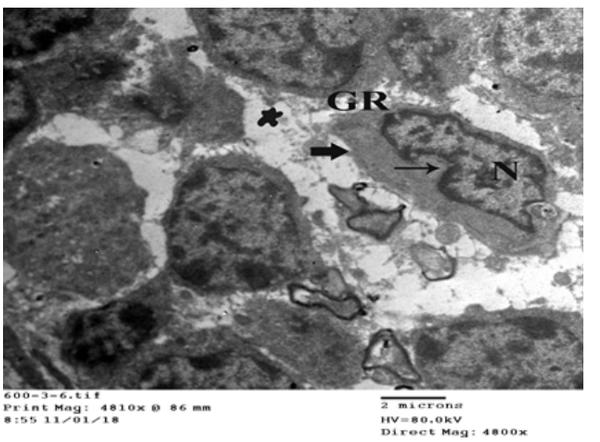


Fig. 24: An electron micrograph of the cerebellar cortex(group III) 3wks treated cerebellum showing group of shrunken granular cells (GR) with shrunken nuclei (N) with irregular nuclear envelope (thin arrow) and more condensed chromatin, the cytoplasmic membrane appear irregular(thick arrow), vaculations appear in between cells (star) (×4810)

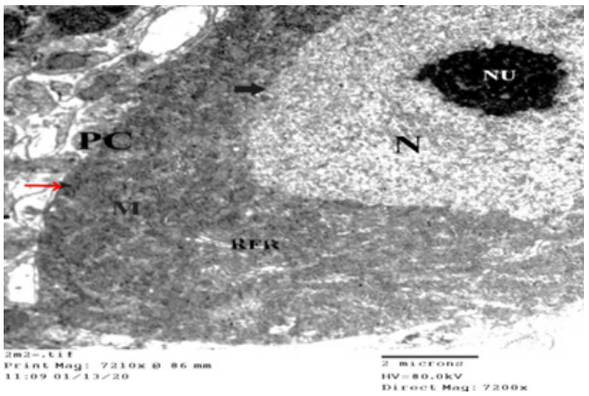


Fig. 25: An electron micrograph of the Purkinje cell of adult control rat cerebellum with its characteristic oval shaped large cell (PC) with well-defined membrane (thin arrow) showing nucleus (N) with well-defined nuclear membrane (thick arrow) and prominent nucleolus (NU) ,the cytoplasm appear with well-defined organelles, the mitochondria (M) with preserved cristae and Rough endoplasmic reticulum (RER)are intact. (x7210)

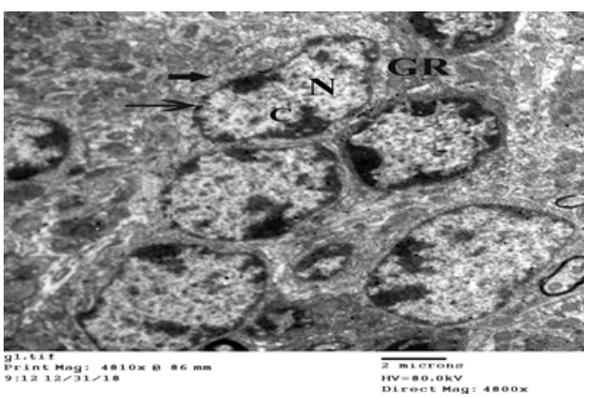


Fig. 26: An electron micrograph of adult control (group I) cerebellum showing group of granular cells (GR) with large size of the nucleus (N) with condensed chromatin(c), and well defined nuclear border (thin arrow) surrounded by thin rim of cytoplasm with intact organelles (thick arrow). (X 4810)

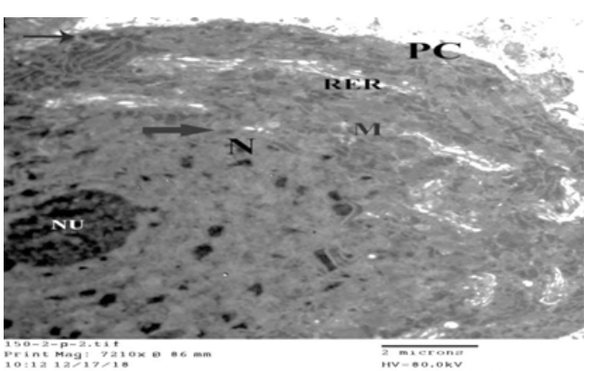


Fig. 27: An electron micrograph of group II of adult treated cerebellum showing shrunken darkly stained Purkinje cell(PC) surrounded by irregular cytoplasmic membrane (thin arrow), the nucleus (N) show condensed chromatin, with ill-defined nuclear envelop (Thick arrow) and prominent nucleolus (NU) the mitochondria appear shrunken(M) and the rough endoplasmic cisterna are dilated (RER). (X7210)

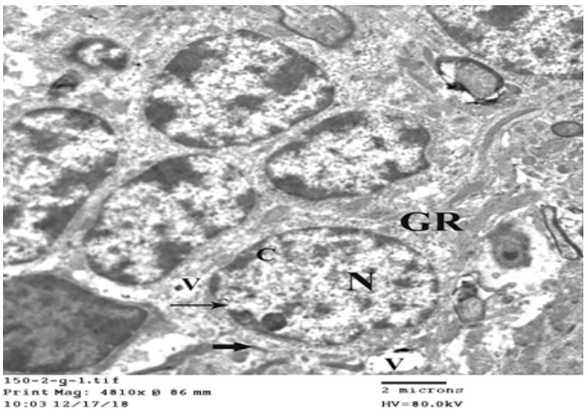


Fig. 28: An electron micrograph of the cerebellar cortex of group II of adult treated cerebellum showing group of granular cells (GR) with large nucleus (N) surrounded by regular nuclear envelope (thin arrow), more condensed chromatin (C), it surrounded by ill-defined cytoplasmic membrane (thick arrow) with vacuoles appear in-between cells(V).(x4810)

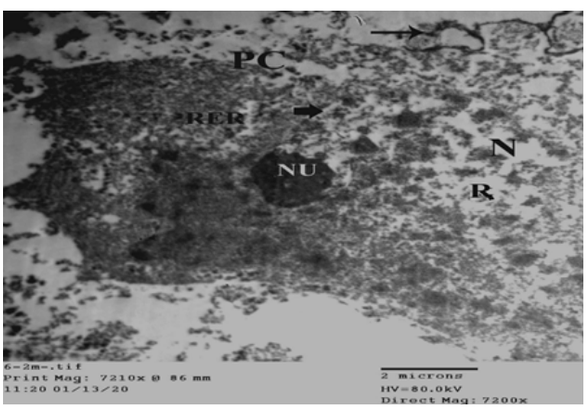


Fig. 29: An electron micrograph of the adult cerebellar cortex of group (III): showing atypical Purkinje cells (PC) with ill-defined cell membrane (thin arrow), destructed nucleus (N), with ill-defined nuclear envelop (thick arrow) and prominent nucleolus (NU), cytoplasm show few rough endoplasmic retinaculum (RER). Rarefication (R) appears within the cytoplasm. (X7210)

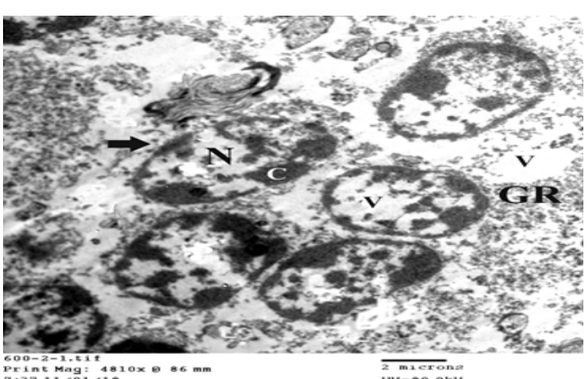
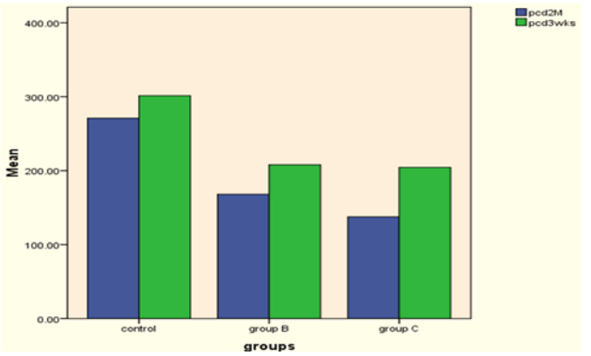


Fig. 30: An electron micrograph of (group III) of adult treated cerebellum showing group of granular cells (GR) with destructed cytoplasmic membrane and organelles (thick arrow), large nuclei (N) with condensed chromatin which mainly peripherally (C) Vacuoles (V) appear within the cytoplasm. (×4810)

Table 1: showing the mean value± standard deviation of the diameter of Purkinje cells both control and treated groups. $P \leq 0.001$ (***) → very highly significant difference

The Purkinje cell diameter	Control	Group B	Group C
3rd weeks	301.4 ± 28.	207.9 ± 46.9***	204.3 ± 38.5***
adult	271 ± 33.19	168.06 ± 38.67***	137.52 ± 32.76***



Histogram 1: showing the mean value± standard deviation of the diameter of Purkinje cells both control and treated groups.

DISCUSSION

The cerebellum is an ideal useful model for studying many aspects of neural development, because each stage of development has a distinct morphology and special histological features with different types of cell[14].

Epileptic pregnant women should continue treatment with AEDs during pregnancy to avoid the potential harmful effect of recurrent seizures that can have significant long term neurological and physical consequences to themselves and their fetuses[15,16].

In the present study light microscopic examination at 1week postnatal rats given pregabalin showed variant changes in the cerebellar cortex which was merely affected at dose 600mg with marked decrease in the thickness of the external granular layer, more cellular molecular layer, degenerated Purkinje cells and non-differentiated internal granular layer. This is in agreement with previous studies[17] in which PGB 600 mg/kg b.wt/day showed the degenerative effect in the Developing cerebellum as antiepileptic drugs affect mainly neurotransmitter systems, blockade N-methyl-D-aspartate receptors or enhanced γ-amino butyric acid inhibition impairs the neurogenesis[18].

At 2 weeks postnatal rats light microscopic examination showed severe neurological abnormalities in pregabalin young rats in both doses with an increase in the thickness of external granular layer , more cellular molecular layer, atypical and pyknotic Purkinje cells these observations are in agreement with the previous results[19] who proved that the increased thickness of external granular layer in 2wk treated group because of pregabalin delays cells migration and differentiation of the granular cells in the developing mouse cerebellum due to its neurotoxic affects as external granular layer of the cerebellum is the site with the most intense proliferative and migratory activity in newborn mammals.

Other studies on pregabalin administration proved that prenatal administration of pregabalin disrupted the architecture and the linear arrangement of the cells in the Purkinje layer[17].

Degenerative changes in the cerebellar cortex of the treated cerebellum and cell death may result from necrosis, pathological or accidental death and could result from extrinsic insults to the cells such as osmotic or toxic traumatic effect of the pregabalin[9].

The present results of the light microscopic examination in age 3 weeks and 2.5 months postnatal rats showed more disturbances in cerebellar cortical layers manifested more in higher dose, Purkinje cells appeared to be mostly affected with losing of their pear shape with ill- defined cytoplasm and nuclei.

These results are in agreement with previous studies[20,21] as they approved by light microscopic examination that the most affected layer of cerebellar cortex in Pregabalin treated cerebellum was the Purkinje cell layer as the cells became shrunken, disarranged with well manifested cell loss.

Pregabalin teratogenicity could be explained by the increase in fetal oxidative stress, as the fetal brain in the rat is more susceptible to increased oxidative stress in comparison to other fetal organs[22,23].

The ultrastructural changes of Purkinje cells of offspring born to pregabalin treated mothers at 3weeks and 2.5 months old rats in the current study confirmed the histological changes noticed by the light microscopic examination; in the form of shrunken and dark appearance of the cells, nuclear changes and irregular cell membranes. These nuclear changes of the Purkinje cells were further accompanied in higher doses of pregabalin by abnormalities in the cytoplasm, which displayed dilatation of the endoplasmic reticulum, destruction of cell membrane and loss of cytoplasmic organelles. Sobaniec[24] explained that the dark appearance of Purkinje cells was probably due to the apoptosis process because these cells displayed markedly condensed karyoplasm and cytoplasm. Also, Chavez-Valdez *et al*.,[25] postulated that the dilation of the endoplasmic reticulum is possible during necrosis

The present ultrastructural examinations of granule cells in the treated groups at 3weeks and 2.5 months old rats showed that the granule cells became shrunken, with marked loss of its cell membrane and cytoplasmic organelles more in high dose of pregabalin, this is in acceptance with previous studies[17,20] where they proved that electron microscopic studies of rats cerebellar cortex treated with pregabalin showed degenerative changes in both Purkinje and granular cells.

Furthermore Pregabalin caused oxidative DNA damage and elevation in some serum biochemical parameters related to the liver and produced oxidative stress in the albino rats[26].

CONCLUSION

Pregabalin administration during pregnancy and lactation causes its marked effect during early postnatal life of the cerebellar cortex and this effect extend till the adult stage of the offspring and these effects are dose dependent.

CONFLICT OF INTERESTS

There are no conflicts of interest.

REFERENCES

1. Standring S., Borley N.R., Collins P. and Alan R. (2008): Gray's anatomy, 40th edition; the anatomical basis of clinical practice, Churchill Livingstone/ Elsevier, cerebellum (20) P: 297-309.
2. Llinas RR, Walton KD, Lang EJ (2004): Ch. 7 Cerebellum. In: The synaptic organization of the brain, 5th Edition (Shepherd GM, ed), pp xiv, 719 p. Oxford; New York: Oxford University Press.
3. Snook L., Paulson L.A., Roy D., Phillips L. and Beaulieu C. (2005): Diffusion tensor imaging of neurodevelopment in children and young adults. Neuroimage; 26(4)1164-1173.
4. Qin L., Wine L., Ahn KJ. and Crenshaw EB. (2006). Genetic analyses demonstrate that bone morphogenetic protein signaling is required for embryonic cerebellar development. J.Neurosci; (26)1896-1905.
5. Morano A, Palleria C, Citraro R, *et al*., (2019) :Immediate and controlled-release pregabalin for the treatment of epilepsy. Expert Review of Neurotherapeutics.; 19 (12): 1167-1177.
6. EtemadL, JafarianAH, MoallemSA. (2015): Pathogenesis of pregabalin-induced limb defects in mouse embryos. J Pharm Pharm Sci.;18:882–889.
7. Menachem E. (2004): Pregabalin pharmacology and its relevance to clinical practice. Epilepsia.: 45 (6): 13-18.
8. Institute Of Laboratory Animal Resources (2011): Commission on Life Science, National Research Council of national academies. Guide for the Care and Use of Laboratory Animals. National Academy press, Washington, D.C, 8th Ed.; 11-55.
9. Salih L, Al-mahdawi1 A, and Al-Salihi A. (2014): Teratological effect of pregabalin drug on the prenatal development of the cerebellum in the albino rat. J. of Recent Scientific Research; 5 (7): 1381-1385.
10. Suvarna SK, Layton C, Bancroft JD. (2013): Bancroft's theory and practice of histological techniques, 7th edition, Churchill Livingstone, El Sevier; 203- 500.
11. Bozzola JJ and Russell LD. (1999): Electron microscopy: principles and techniques for biologists, second edition, Boston, Jones and Bartlett Publishers; pp 100-124
12. Maricich SM, Soha J, Trenkner E, Herrup K (1997) Failed cell migration and death of Purkinje cells and deep nuclear neurons in the weaver cerebellum. J Neurosci 17: 3675–3683.
13. Dean AG, Arner TG, Sunki G, Sangam S, Friedman R, Lantinga M, and Diskalkar S.(2000): Epi-info version 1 for the year 2000. A Database and Statistics Program for Public Health Professionals CDC. Georgia, USA, :1-191
14. Eilers J., Plant T.D., Marandi N. and Konnerth A. (2001): GABA-mediated Ca+2 signaling in the developing rat cerebellar purkinje neurons, J. Physiol.; 536(2):429-437.
15. Gilboa S.M., Broussard C.S., Devine O.J., Duwe K.N., Flak A.L., Boulet S.L., Moore C.A., Werler M.M. and Honein M.A. (2011): Influencing clinical practice regarding the use of antiepileptic medications during pregnancy: Modeling the potential impact on the prevalences of spina bifida and cleft palate in the United States. Am J Med Genet Part C Semin Med Genet 157:234–246.
16. Ban L., Fleming K., Doyle P., Smeeth L., Hubbard R., Fiaschi L. and Tata L. (2015): Congenital anomalies in children of mothers taking antiepileptic drugs with and without Periconceptional high dose folic acid use: a population-based cohort study.PLoS ONE 10(7).
17. Yadav P., Shresths S., Kushwaha R.P., Jha C.B., Rauniyar G.P. and Shah R. (2018)S: Effect of folic acid in prenatal pregabalin-induced cerebellar changes in the Swiss Albino Mice, IJTA, (35);19-25.
18. Stefovska V., Uckermann O., Czuczwar M., Smitka M., Czuczwar P., Kis J., Kaindl A., Turski L., Turski W. and Ikonomidou C. (2008): Sedative and anticonvulsant drugs suppress postnatal neurogenesis. Ann. Neurol. 64; 434–445.
19. Hatten M.E. and Heintz N. (1995): Mechanisms of neural patterning and specification in the developing cerebellum. Annu. Rev. Neurosci:(18)385-408.
20. Swillam A., Shalaby S., Eid E. and Sarg N. (2018): the prenatal and postnatal effects of pregabalin on the cerebellar cortex of the albino rat and the possible protective effect of l-carnitin, Banha University Journal. p182
21. Kim J., Kondratyev A., and Gale K. (2007): Antiepileptic drug- Induced neuronal cell death in the immature brain: effects of Carbamazepine, Topiramate and Levetiracetam as monotherapyvsPolytherapy. J.Pharmacol. Exp. Ther. 322(1): 165-173.

22. Danielsson B, Danielsson C and Nilsson F.(2007):Embryonic cardiac arrhythmia and generation of reactive oxygen species: common teratogenic mechanism for IKr blocking drugs. Repro. Toxicol.; 24 (1): 42-56.

23. Banji D, Banji OJ, Abbagoni S, Hayath MS, Kambam S, Chiluka VL. (2011): Amelioration of behavioral aberrations and oxidative markers by green tea extract in valproate induced autism in animals. Brain Res;1410:141–51.

24. Sobaniec-Lotowska M. (2001): Ultrastructure of Purkinje cell perikarya and their dendritic processes in the rat cerebellar cortex in experimental encephalopathy induced by chronic application of valproate. Int. J. Exp. Pathol.; 82 (6): 337-348.

25. Chavez-Valdez R, Flock D, Martin J. *et al*., (2016): Endoplasmic Reticulum pathology and stress response in neurons precedes programmed necrosis after neonatal hypoxia-ischemia. Int. J. Dev. Neurosci.; 48: 58-70.

26. Kamel MA (2016): Study on DNA damage and oxidative stress and some biochemical alterations of long term administration of alpha-2 delta (A2-Δ) ligand pregabalin and the possibility of Zingiberofficinale in ameliorating these effects in rats. World J Pharm Res 5:1528–1545

الملخص العربى

تأثير الجرعات المختلفة من عقار البريجابالين على نمو القشرة المخيخية فى فترة مابعد الولادة فى الفئران البيضاء

سلوى محمد عويس[٢]، زهراء محمد اسماعيل[٢]، مصطفي عبد الخالق عبد اللاه[١]

[١]اقسام التوليد وأمراض النساء، [٢]قسم التشريح وعلم الاجنة- كلية الطب، جامعة سوهاج

مقدمة: عقار البريجابالين هو دواء عن طريق الفم يستخدم لعلاج آلام الأعصاب واضطراب القلق العام والصرع وكعلاج للنوبات الجزئية. لا يكتمل نمو المخيخ أثناء فترة الحمل فقط ولكنه يستمر بعد الولادة حتى نضوج المخيخ (تطور ما بعد الولادة).

الهدف من العمل: دراسة التأثير المحتمل للبريجابالين على نمو ما بعد الولادة للقشرة المخيخية فى الفئران البيضاء.

المواد والطرق المستخدمة: تم إجراء الدراسة الحالية على ٦٠ من الفئران الحوامل مقسمة إلى ثلاث مجموعات. المجموعة أ: تشمل ٢٠ نسلًا من ٢٠ أمًا ضابطة ، المجموعة ب: تشمل ٢٠ نسلًا من ٢٠ أمًا تم علاجهم عن طريق الفم بعقار البريجابالين بجرعة ١٥٠ مجم / كجم ، المجموعة ج: تشمل ٢٠ نسلًا من ٢٠ أمًا تم علاجهم بـعقار البريجابالين عن طريق الفم بجرعة ٦٠٠ مجم / كجم. في المجموعات الثلاث تم التضحية بالفئران في فترة ما بعد الولادة (العمر ١ ، ٢ ، ٣ أسابيع) بالإضافة إلى عمر البالغين (٢,٥ شهر) ، تم فتح الجماجم وإزالة المخيخ ومعالجته للفحص بالمجهر الضوئي والمجهر الإلكتروني النافذ

النتائج: إعطاء عقار البريجابالين أثناء الحمل والرضاعة يأثر بشكل ملحوظ خلال الفترة المبكرة ما بعد الولادة ، ويمتد هذا التأثير حتى مرحلة النضوج على شكل زيادة في الطبقة الحبيبية الخارجية (بشكل رئيسي في الأسبوعين الثاني والثالث) ؛ سمك أقل للطبقة الجزيئية في جميع المجموعات المعالجة ؛ خلايا بركنجى اصبحت متقلصة وغير طبيعية وبالإضافة الى تأخر تمايز الطبقة الحبيبية الداخلية

الخلاصة: إعطاء عقار البريجابالين قبل وبعد الولادة بجرعة منخفضة ومرتفعة تسبب في فقدان المكونات الخلوية ، وتشوهات الخلايا القشرية المخيخية بطريقة تعتمد على الجرعة.