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Effect of pre and postnatal administration of pregabalin on the granule cells of the cerebellar cortex of albino rats (An Electron microscopic study)

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ABSTRACT

Introduction: the cerebellum organogenesis in the mammals takes place a long long period of the development from the embryogenesis to the postnatal time after delivery to reach the mature form morphologically and physiologically, therefore these stages are very critical and sensitive to the growth of the cerebellum.

Pregabalin is an oral medication that used to treat neuropathic pain, fibromyalgia, generalized anxiety disorder, and epilepsy as a therapy for partial seizures

Aim of the work: The present study was carried out to study the effect of low therapeutic dose and high therapeutic dose of pregabalin administration in pre and postnatal periods on granule cells of the cerebellar cortex of albino rats.

Subjects and methods: The study included a total of 15 offspring 15 pregnant female albino rats were subdivided into three groups: **Group A:** Includes offspring's of 5 control mothers received distilled water, **Group B:** Includes offspring's of 5 mothers treated with pregabalin at the dose of 150mg/kg, **Group C:** includes offspring of 5 mothers treated with pregabalin at the dose of 600mg/kg, the offspring are sacrificed at 2.5 months, Cerebellar specimen were processed for transmission electron microscopic assessment.

Results: ultrastructure studies of granule cells in group B offspring were altered than the control group, the cytoplasmic membrane was ill-defined and some vacuoles were presented, the nucleus showed disturbed chromatin.

Ultrastructural studies of granule cells in group C offspring showed shrunken granule cells, ill-defined cytoplasmic membrane, destructed cytoplasmic organelles and marked cytoplasmic vacuoles, the nucleus showed heterochromatic chromatin.

Conclusion: pre and postnatal administration of pregabalin in both low and high doses proved the loss of cellular components, distortions of cerebellar cortical cells that may be an effect on the physiological functions of the cerebellum.

Keywords: Cerebellum,pregabalin,postnatal,transmission Em.

Introduction:

The cerebellum is the second largest part of the brain, lies behind the pons and medulla, the cerebellum is an ideal model for studying many aspects of neural development because each stage of development has a special morphological and histological features with different types of cells, the process of intrauterine cerebellar development (prenatal development) continues after

delivery to maturation of the cerebellum (postnatal development). ***(1)***

Pregabalin is an oral medication used to treat neuropathic pain, fibromyalgia, generalized anxiety disorder, and epilepsy as a therapy for partial seizures. ***(2)***

Pregabalin is beneficial in the treatment of epilepsy when added to other antiepileptic drugs in the control of partial seizures. ***(3)***

Pregabalin acts by preventing calcium influx and the next release of excitatory neurotransmitters this is the cause of its neurotoxic effects because these ion channels and neurotransmitter systems in the brain are responsible for the regulation of processes important for the brain organogenesis. ***(4)***

Materials and methods:

In this study, a total of 15 adults pregnant female albino rats their weight range from 200-250g were used. The animals were brought from the animal house of the Assiut faculty of medicine. They were reared under-the standard conditions of feeding, light-dark ratio, and temperature, in that faculty of medicine animal house.

The rat was kept in plastic cages in the ratio of 1:2 males and females. Females were examined for vaginal plugs (an indication of the presence of sperm in the vagina) and separated for the experimental protocol and their gestational days were recorded.

The animals were subdivided into three groups:

Group A: Includes 5 offspring of 5 control mothers who received distilled water.

Group B: Includes 5offspring of 5 mothers treated with pregabalin at the dose of 150mg/kg by oral tube daily starting from the 1st day of the positive vaginal smear till birth and during whole period of lactation (up to postnatal day 21) according to ***(5)***

Group C: Includes 5offspring of 5 mothers treated with pregabalin at a dose of 600mg/kg, by oral tube daily starting from the 1st day of the positive vaginal smear till birth and during the whole period of lactation (up to postnatal day 21) according to ***(5).***

Drug, dosage and administration:

Pregabalin capsules (Lyrica®) has obtained from the pharmaceutics and used in the effective doses: 150,600mg/kg, according to the doses used in several clinical cases in humans, depending on the recommended documentation of the Pfizer company, and the local pharmacies.

Methods:

The rats' offspring from each of the groups were randomly selected, sacrificed at age of 2,5 months postnatal, by intramuscular IM injection of a mixture of Ketamine (90 mg/kg body weight) and Xylazine (10 mg/kg body weight) then their skulls were opened and their cerebellar tissues were taken for transmission electron microscope study.

Ultrastructural study of the granule cells of the cerebellar cortex was done through the preparation of ultrathin sections stained with uranyl acetate, lead citrate, examined, and photographed by Jeol-JEM-100 CXII electron microscopy.

Morphometric and statistical analysis:

Estimation of the diameter of granule cell nuclei was done by using (digitizer version 3.7.2005-2010) Medical software in the anatomy department at Sohag University. Statistical analysis of the data was done using spss software version 16.variable were represented by (mean ±standard deviation of

the mean) independent t-test to compare mean of a variable between different groups. Finally, the significance was considered according to the level of significance (P-value) as follows:

P > 0.05 (NS) → No significant difference.

P ≤ 0.05 (*) → Significant difference.

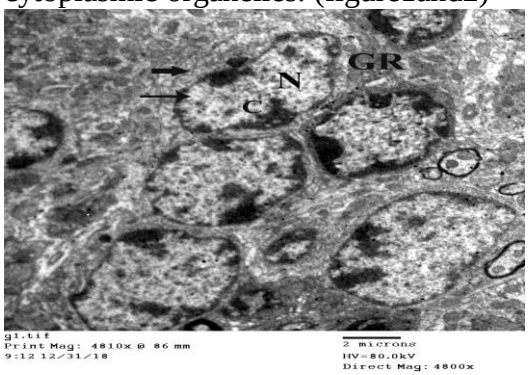
P ≤ 0.0l (**) → High significant difference.

P ≤ 0.001 (***) → Very high significant difference.

RESULTS:

Group A (Control):

The granule cells appeared more or less equal in size with a large nucleus containing clumps of chromatin and the thin layer of cytoplasm with intact cytoplasmic organelles. (figure1and2)



Figure(1): Electro micrograph of adult control(group A) cerebellum showing a group of granular cells (GR) with large size of the nucleus (N) with condensed chromatin(c), and show well defined nuclear border (thin arrow) surrounded by a thin rim of cytoplasm with intact organelles (thick arrow). (X 4810)

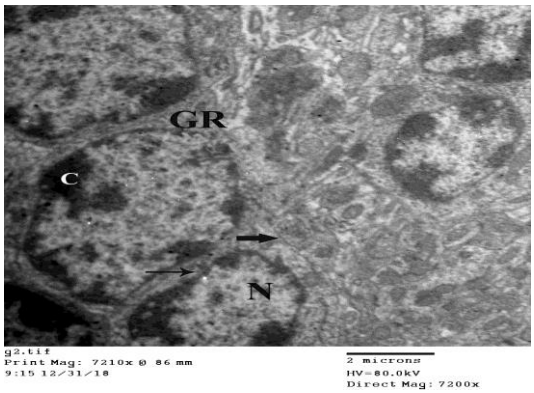


Figure (2): the magnified image of the previous figure show large size of the nucleus (N) with a well-defined nuclear border (thin arrow) surrounded by a thin rim of cytoplasm with intact organelles (thick arrow). (x7210)

Group B (150mg pregabalin):

The granule cells showed ill-defined cytoplasmic membrane with some vacuoles were presented, the nucleus showed disturbed chromatin. (figure3and4)

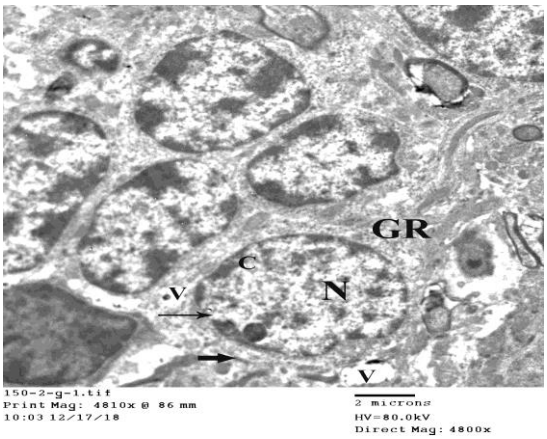


Figure (3): An electron micrograph of the cerebellar cortex of (group B) treated cerebellum showing group of granular cells (GR) with large nucleus (N) surrounded by the regular nuclear envelope (thin arrow), disturbed chromatin (C), it surrounded by an ill-defined cytoplasmic membrane (thick arrow) with some vacuoles (V). (x4810)

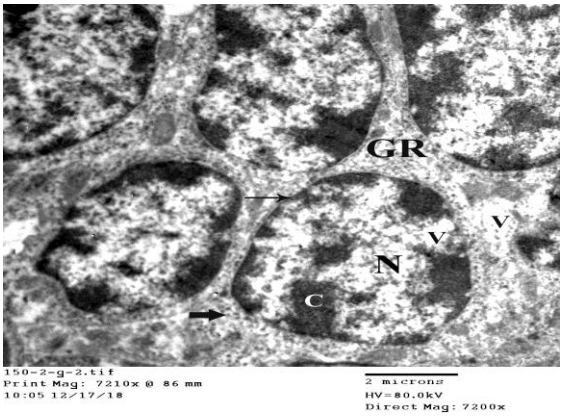
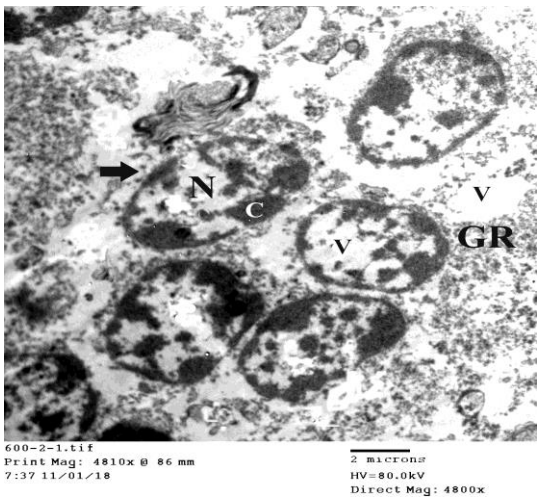


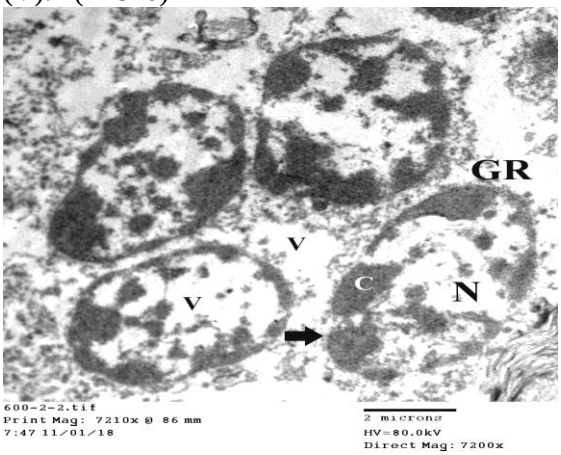
Figure (4): A magnified image showing granular cells (GR) with large nucleus (N) with the regular nuclear envelope (thin arrow), peripheral heterochromatin (C), it surrounded by an ill-defined cytoplasmic membrane (thick arrow) with some vacuoles in both nucleus and cytoplasm (V). (X7210)

Group C (600mg pregabalin):

The granule cells were shrunken, ill-defined cytoplasmic membrane destructed cytoplasmic organelles, and marked cytoplasmic vacuoles, the nucleus showed disturbed chromatin. (figure5and6)



Figure(5): Electro micrograph of group C treated cerebellum showing group of granular cells (GR) with destructed cytoplasmic membrane and organelles (thick arrow), large nuclei (N) with the discontinued nuclear envelope (arrow) , chromatin crowded peripherally (C) with marked cytoplasmic and nuclear vacuoles (V). (×4810)



Figure(6): the magnified image of the previous picture showing a group of granular cells (GR) with destructed cytoplasmic membrane and organelles (arrow), large nuclei (N), chromatin crowded peripherally (C) with marked cytoplasmic and nuclear vacuoles (V). (X7210)

Morphometric study:

The mean value of the nuclear diameter of granule cells in group B was (470.8), which is no significant difference (P>0.05) compared with the control group.

The mean value of the nuclear diameter of granule cells in group C was (370.65), which is significantly decrease (P≤0.000) compared with the control group.

	Control	Group B	p-value
The nuclear diameter of granule cells.	518.82±107.2	470.8±71	P>0.05

Table (1): showing the mean value± standard deviation of the nuclear diameter of granule cells in both control and group B

P> 0.05 → no significant difference.

	Control	Group C	p-value
The nuclear diameter of granule cells.	518.82±107.2	370.65±83(***)	P≤0.000

Table (2): showing the mean value± standard deviation of the nuclear diameter of granule cells in both control and group C

P≤0.000 (***) → Very high significant difference.

DISCUSSION

The maternal exposure to acute or chronic illness or exposure to some drugs or toxins during the early trimesters of pregnancy causes serious changes in central nervous system embryogenesis especially the cerebellum.***(6)***

Pregabalin has been passing easily the blood-brain barrier and placenta in preclinical studies in rats, mice, and monkeys. Thus the drug can cause an effect on the development of the central nervous system. ***(5)***

In the present study, the albino rats were chosen to study the effect of low and high doses of pregabalin administration pre and postnatal on the granule cells of the cerebellum. The present study showed that in 150mg(low therapeutic dose) pregabalin treated rats the granule cells had ill-defined cytoplasmic membrane with some vacuoles were presented, the nucleus showed disturbed chromatin, these results were in acceptance with ***(7)*** noted electron microscopic degenerative changes of both Purkinje and granular cells in Pregabalin treated group.

The present study also showed that in 600mg (higher limit of therapeutic dose) pregabalin treated rats the granule cells became shrunken, with marked loss of its cell membrane, cytoplasmic organelles and destructed nuclear envelope with marked chromatin destruction, this was in acceptance with ***(8)*** reported that administration of gabapentenoid as pregabalin and topiramate during pregnancy promotes hippocampal and cortical malformations in a dose-dependent manner.

pregabalin mechanism of action is by suppressing calcium influx and the following release of excitatory neurotransmitters, these ion channels and neurotransmitters are essential for the regulation of processes of brain development ***(4)***, as proved by ***(9)*** they revealed that the neurotoxic effect of many antiepileptic drugs in rodents cause neurodegenerative changes in the developing rat brain.

Cellular toxic action of pregabalin also proved by ***(10)*** they reported that pregabalin can effect on the growth of follicles in the ovary via Calcium channels as in the mechanism of its action, Calcium ions are one of the factors required as well as follicular stimulating hormone and the growth factors to complete growth and differentiation of follicles.

The important factor controlling the susceptibility of the brain to antiepileptic drugs is the timing of exposure proved by ***(11)***. It has been reported that administration of any teratogens from gestational age 7 to gestational age 13, the most critical period of gestation, caused a reduction in a cell population, decrease in thickness in some zones, loss of intermediate zone and discontinuation of the marginal zone in the cerebral cortex***.(9)***

Conclusion: pre and postnatal administration of pregabalin in both low and high doses proved the loss of cellular components, distortions of cerebellar cortical layers that may be an effect on the physiological functions of the cerebellum.

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