WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 6.805
Volume 5, Issue 10, 33-42. Research Article ISSN 2277– 7105

EFFECT OF LEVETIRACETAM ON THE CEREBRAL CORTEX OF NEWBORN RAT

Ramih A. Nasser[1], Nahla A. AL-Bakri[2], Mohammad Oda Selman[1*]

[1]High Institute of Infertility Diagnosis and ART, Al-Nahrain University.

[2]Collage of Education for Pure Science-IBN Al-Haitham/Baghdad University.

Article Received on
30 July 2016,

Revised on 19 Aug 2016,
Accepted on 09 Sap 2016

DOI: 10.20959/wjpr201610-7073

***Corresponding Author'**
Mohammad Oda Selman
High Institute of Infertility Diagnosis and ART, Al-Nahrain University.

ABSTRACT

Background: Levetiracetam is a member of the new antiepileptic drugs and has a broad spectrum effect, used as an adjunctive therapy in addition to monotherapy in the treatment of partial onset-seizures. The effect of levetiracetam on the development of embryo nervous system after maternal exposure during pregnancy has not been identified. **Objective:** to evaluate the effect of antiepileptic drug, levetiracetam (LEV) within its therapeutic dose 350mg/Kg body weight on albino female rat to clarify its effect on the developing cerebral cortex histologically. **Material And Methods:** Ten pregnant female rats were separated into two groups, control group and experimental group. They were obtained from the animal house of the high institute of infertility diagnosis and assisted reproductive technologies/Al-Nahrain university. They were maintained in environmentally controlled room at a temperature of 21–28±4C°, 40–60% humidity, 12 hours light-dark cycle, in a noise free environment. Oral administration of 350mg/Kg of LEV to the experiment group while physiologic saline was given to control group. **Results:** microscopic assessment of the cerebral cortex defects in the cerebrum of the treated group when compared with the control group. There was disorganization of the cortical layers where boundaries were dimmed, the depth of the six layers were overlapping, decreased proportion of the stellate cells in the external granular layer therefore, reducing layer outline, vascular congestion and hemorrhage. Furthermore, observation shows cellular degeneration, necrosis, and nucleus karyorrhexis. **Conclusion**: this study demonstrate that they must take care from giving Levetiracetam to pregnant female because it induces histological changes in the brain of the newborn rat.

KEYWORDS: Levetiracetam, antiepileptic drugs, brain vascular congestion.

INTRODUCTION

Levetiracetam is a second generation of antiepileptic drugs (AEDs), it is chemically unrelated to other AEDs and it is alpha-ethyl analogue of piracetam.[1] It is widely used and well tolerated agent, licensed as adjunctive therapy in the management of partial onset seizures, also indicated in other epilepsy syndrome such as idiopathic generalized epilepsies, recently LEV accepted and used as monotherapy. With this broad profile, it is widely dependent in women with epilepsy of childbearing potential and during pregnancy period, however information on the adverse effect outcome has not been well established.[2,3] LEV is highly soluble in water and absorbed rapidly and completely after oral administration and its metabolism is independent of any cytochrome P450 enzymes system rather that Lev enzymatically hydrolyzed in blood vessels[4], also LEV has slightly affinity to bind to plasma proteins, while the excretion of Lev is through renal system where 66% of the dose is excreted unchanged then the its elimination is proportionally to creatinine clearance.[5]

The mechanism of how LEV exerted its anticonvulsive effect does not seem to share any means of the mechanism in the older AEDs, rather that it possesses a novel mechanism giving it a unique pharmacological profile, the unique LEV-binding site in the brain is an integral membrane glycoprotein (Synaptic vesicle protein 2A isoform) presented on all synaptic vesicles of the brain which has a crucial role in regulation the vesicle function.[6,7]

Epilepsy is a common neurological disorder that affects people of all ages, women with epilepsy (WWE) who receive AEDs though their pregnancy period to overcome the risk of having seizure are most likely to encounter developmental disorders in their offspring such as minor anomalies, intrauterine growth retardation, low birth weight, cognitive dysfunction, developmental delay, microcephaly, major congenital malformations, and infant mortality.[8,9] The majority of WWE have a normal pregnancy outcome, Careful management of pregnancy in WWE is crucial to minimize these risks.[10]

MATERIAL AND METHODS

Ten adult female rats were randomly divided into two groups, five on each. Vaginal smear was performed to all female rat to investigate the stages of estrus cycle, on the estrous phase then mates with mature healthy males to induce conception. The occurrence of vaginal plug or sperm positive vaginal smear was indicated as a successful mating and the day follows

considered as day one of pregnancy.[11] levetiracetam 350mg/Kg body weight rat/ day was administrated orally by gavage to the pregnant female rats of the treated group from day one of pregnancy and continued to day seven postnatally. In the contrary, physiological saline was administrated to control group and following the same procedure of the first group. Thirty newborns from each group were euthanized and brains were dissected, Bouin's solution was used as a fixative. Histological examination to the cerebral cortex was done via paraffin sectioning procedure as follows: Bouin's fixative for 24 hours, washing until the yellow color fainted, dehydration with alcohol 70%, 95% for 6 hours, 100% for 2 hours, infiltration with hot paraffin for 3 hours, and embedding, sectioning[12] Paraffin sections were stained with Haematoxylin/ Eosin (H&E), the specimens were examined under light microscope.[13]

RESULTS

The brain specimens in both groups were oriented to obtain sagittal sections for microscopic examination, the results of the cerebral cortex of treated group were compared with the normal that obtained from newborns of females in the control group. The normal cerebral cortex shows six distinct cortical layers delimited between the meninges and the corpus callosum, the first layer (I) is the molecular layer contains few scattered cells of Cajal, the second layer (II) is the external granular composed of densely packed small pyramidal neurons, the third layer (III) is the external pyramidal that contains small and medium pyramidal neurons, the fourth layer (IV) is the internal granular which consists of small granular cells, the fifth layer (V) is the internal pyramidal where large pyramidal neurons are found, and the sixth layer (VI) is the multiform that consists of few large pyramidal neurons, many small spindle-like pyramidal neurons and multiform neurons, the results of the treated group show disorganization in the extension and depth of the six layers where boundaries were fainted and imbricated. (Fig.1) The molecular layer displays irregularity in its extension and appeared folded instead of striated as in the control group. (Fig.2)

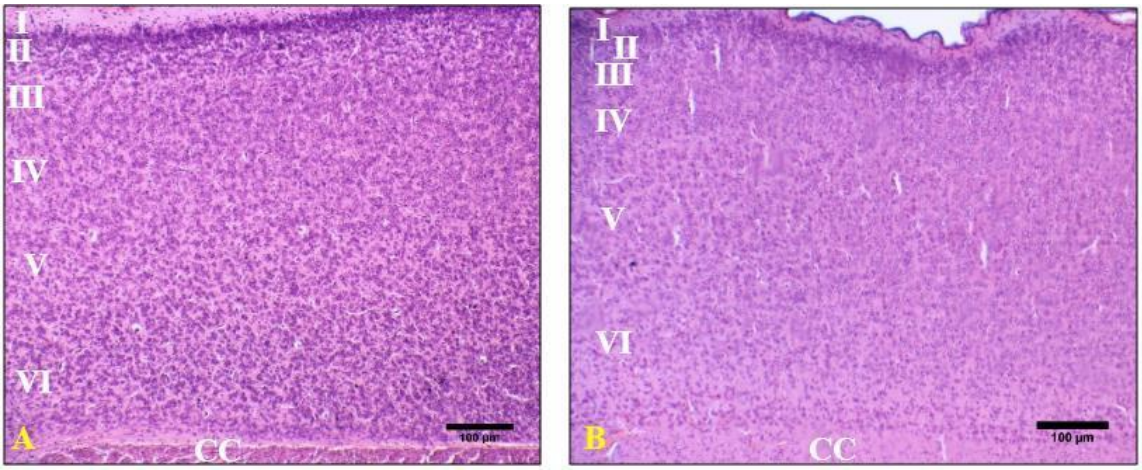

Fig.1: Sagittal sections in the cerebral cortex of newborn rat in the 7th day of age, show the cortical layers (I-VI) of the cerebrum. A) control group, B) treated group with levetiracetam (350mg/kg rat body weight/day). Notice the disorganization of the cortical layers of the treated group in comparison to the consistency in the control group, corpus callosum (CC). (H&E), 10X.

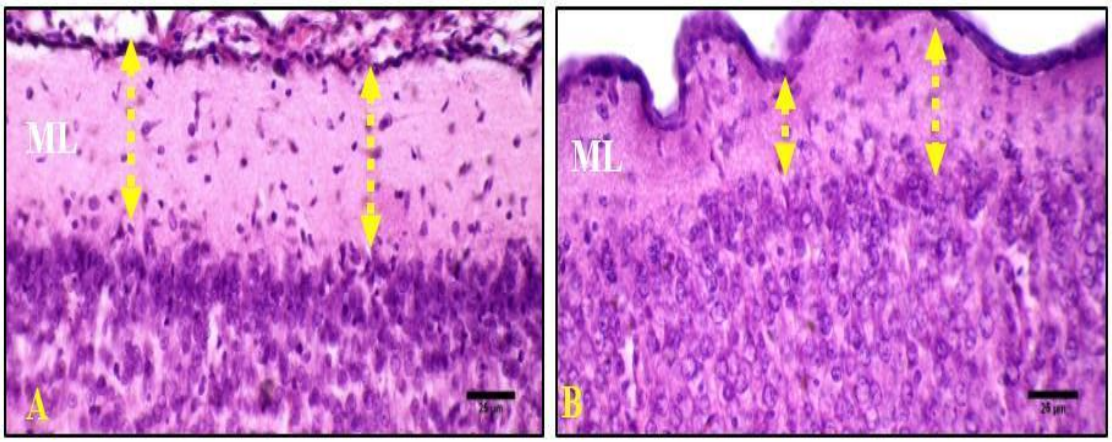

Fig.2: Sagittal sections in cerebral cortex of rat newborn in the 7Th day of age shows the molecular layer. A) control group, notice normal extension of the molecular layer, B) treated group with levetiracetam (350mg/kg rat body weight/day), notice aberrant extension of the molecular layer, all indicated by the yellow doted double arrow, molecular layer (ML), (H&E), 40X.

The external granular layer shows decline in the differentiation of the cellular components as a layer of densely packed cells and superseded by less proportion and loosely appearance and therefore reducing boundaries, also there was cellular degeneration, pyknotic nucleus, cell shrinkage, and congested blood vessel. (Fig.3)

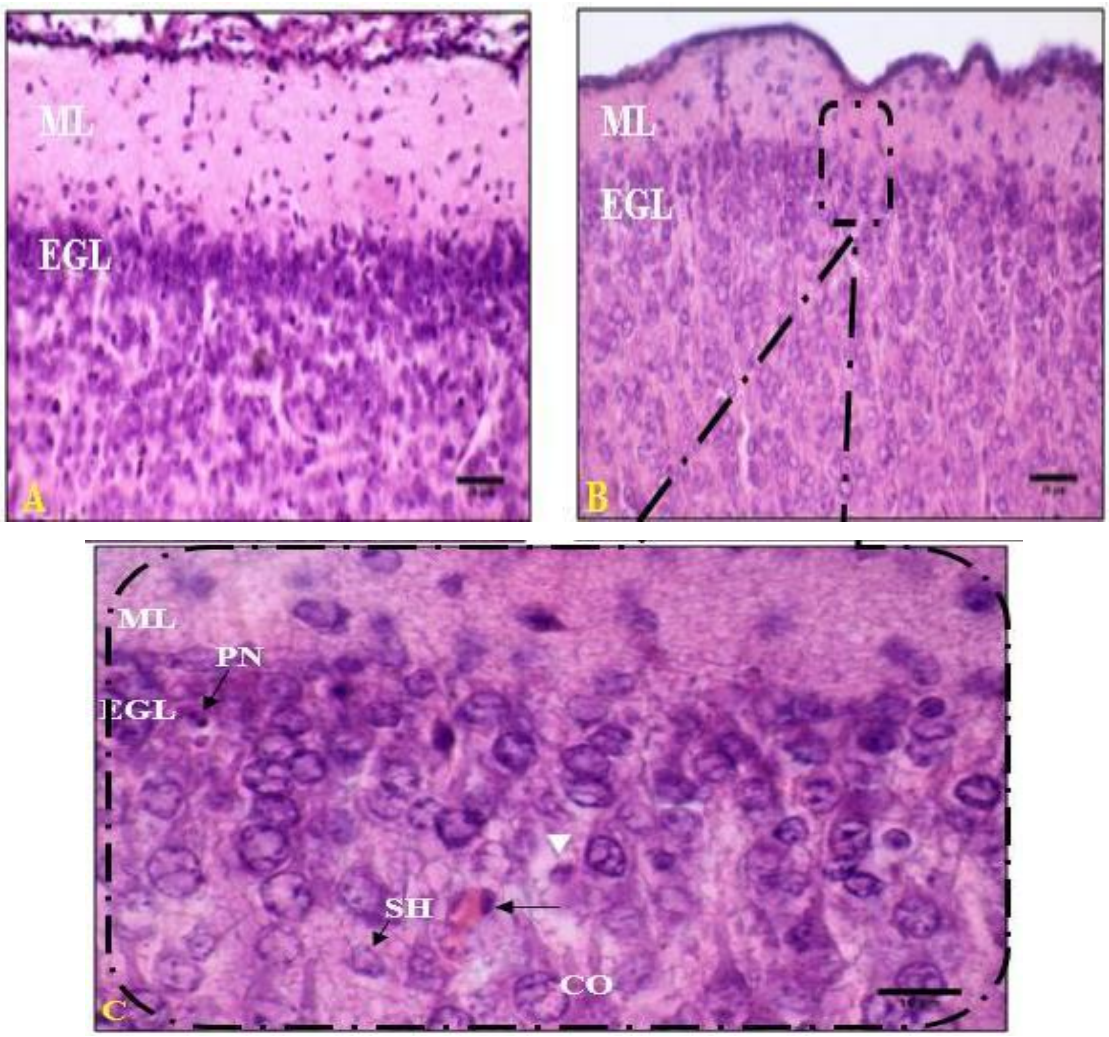

Fig 3: Sagittal sections in cerebral cortex of rat newborn in the 7Th day of age shows the external granular layer. A) control group, notice the appropriate number and distribution of stellate cell and normal appearance of the external granular layer, 40X, B) treated group with levetiracetam (350mg/kg rat body weight/day), notice the inconspicuous external granular layer, 40X, C) magnified view of the doted rectangle in (B), notice fewer cell number of scanty EGL, 100X. molecular layer (ML), external granular layer (EGL), degenerated cell (arrowhead), pyknotic nucleus (PN), cell shrinkage (SH), congested blood vessel (CO), (H&E).

Cells of the cerebrocortical layers (III-VI) in the control group of rat newborn in the seventh day of age were normal in shape with conspicuous nucleus and prominent nucleoli, and cells appears normal chromophilic with H&E stain. (Fig. 4)

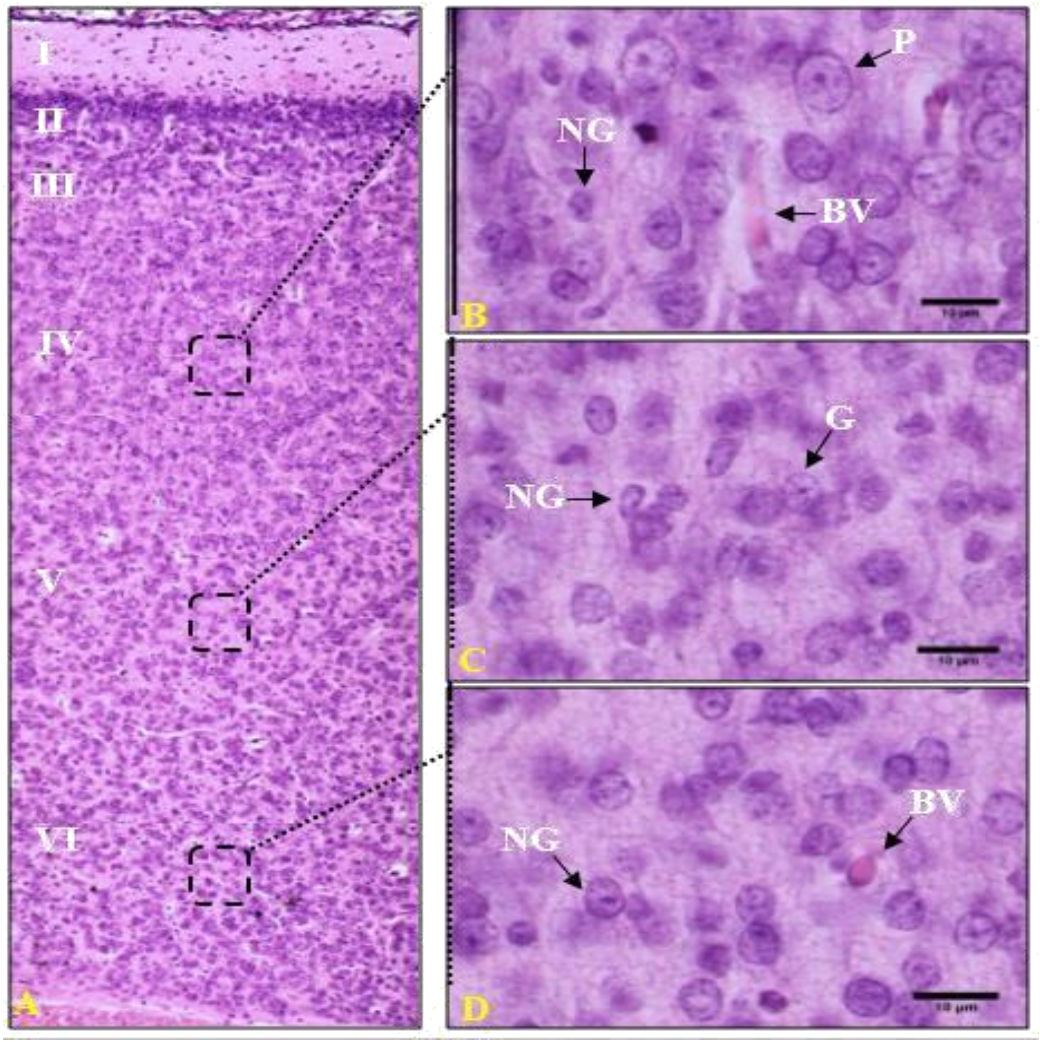

Fig 4: Sagittal sections in the cerebral cortex of rat newborn in the 7Th day of age of control group shows cortical layers of the cerebrum. A) cerebral cortex, 40X, B) magnified view of the rectangle in cortical layer IV, 100X, C) magnified view of the rectangle in cortical layer V, 100X, D) magnified view of the rectangle in in cortical layer VI, 100X. Notice: pyramidal neuron (P), granule neuron (G), neuroglia (NG), blood vessels (BV), (H&E).

Cells of the cerebrocortical layers (III-VI) in the treated group of rat newborn in the seventh day of age shows several cellular changes distributed along the layers when compared with control group, where several cells were fainted and not well recognized, granulovacuolar degeneration, irregular, pyknotic and karyolitic nucleus, necrotic cell, cell with scanty cytoplasm, and degenerated cells. (Fig.5)

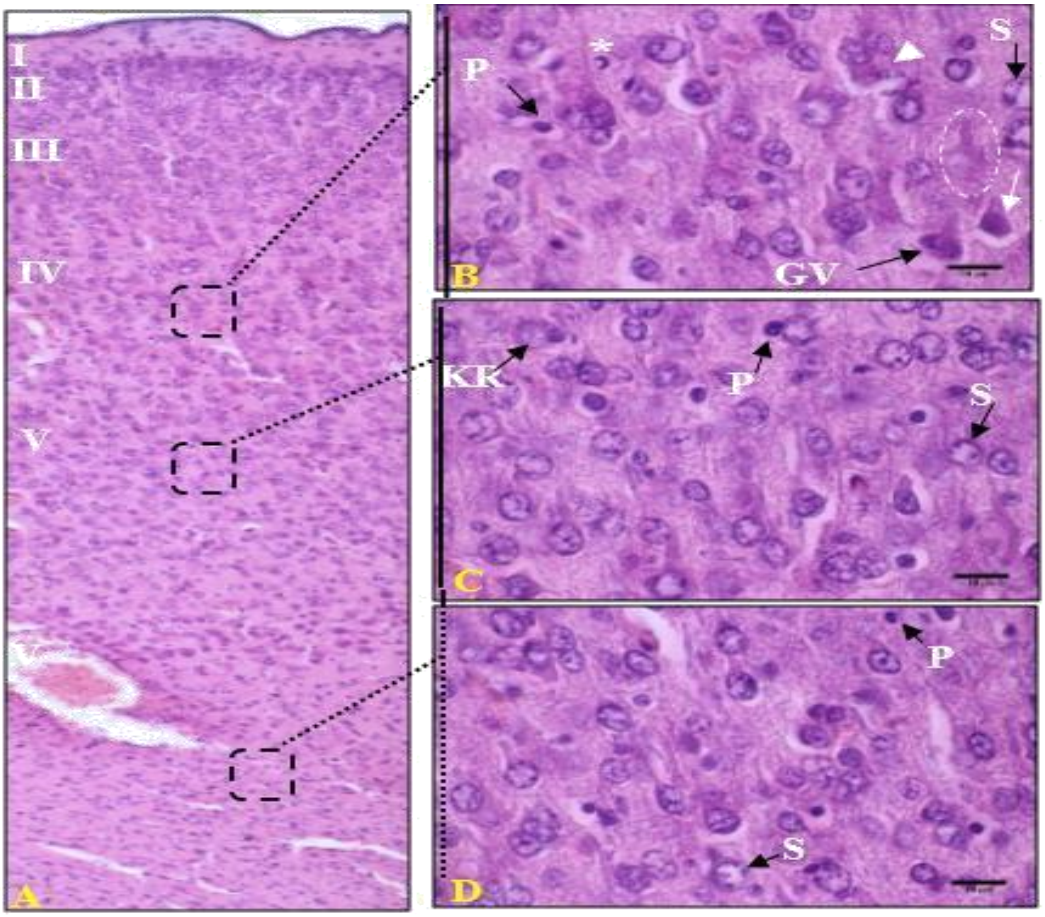

Fig.5: Sagittal sections in the cerebral cortex of rat newborn in the 7Th day of age of treated group with levetiracetam (350mg/kg rat body weight/day) shows cortical layers of the cerebrum. A) cerebral cortex, 40X, B) magnified view of the rectangle in cortical layer IV, 100X, C) magnified view of the rectangle in cortical layer V, 100X, D) magnified view of the rectangle in in cortical layer VI, 100X. Notice: pyknotic nucleus (PN), scanty cytoplasm (SC), granulovacuolar degenerated cell (GVD), Karyolytic nucleus (KR), necrotic cell (arrow), degenerated cell (arrowhead), irregular nucleus (star), faint cell (doted oval shape), (H&E).

DISCUSSION

The present study examined the effect of LEV (350mg/Kg body weight rat/day) on the developing rat newborn day 7 cerebellar cortex via histological sections with principle stain (H&E). The development of the cerebral cortex of the brain starts prenatally where proliferation and migration of cells are predominant with less observed differentiation and maturation, while it is predominant in late prenatal and postnatal life.[14] Many studies described that steps of neuronal formation in mammals are the same in human and informed that postnatal exposure to AEDs has adverse effect in developing a cerebellar

degeneration.[15,16] The current study clearly showed that the administration of LEV (350mg/Kg body weight rat/day) to pregnant female rat induce neuronal death in the cerebral cortex of the offspring, where necrotic cells, pyknotic and karyolitic nuclei were observed, when irreversible stage of injury reached by a neuron exposed to an insult it undergoes a morphological changes recognized as cell death.[17] AEDs are reported to induce necrotic cell death where most of the classic AEDs induce large-scale neuronal death in some specific regions of the neonatal brain.[18] Our result is agree with a study in 2011 on rat brain and reported that phenobarbital exposure results in a significant increase in cell death in all brain region.[19] This result in contrast with a study in 2007 tested the potential neurotoxicity of three AEDs (carbamazepine, Topiramate, and Levetiracetam) on the developing rat brain, the author administrated the medications to neonate rat at age 7,8 days as an intraperitoneal injection 24 hours before sacrificing, and he suggested that LEV did not induce cell death, nor did it exacerbate phenytoin-induced neurodegeneration.[20] Our study shows several histological changes in the cerebral cortex recognized as congested blood vessels, neuronal cell degeneration, and shrunken neurons. Blood vessel congestion is a term used to describe the accumulation of blood cells in a dilated vessels of an organ, the term is described by Mohan H. as a sign of disturbances in the volume of circulating blood[21], our results is in agreement with a research investigate the effect of AED Oxcarbazepine on rat neonate, and our finding exacerbate the result of testing LEV on internal organ of adult rats.[22, 23] Also we agree in the point of neuronal cell degeneration with the assumption that repeated administration of ketamine increase neuronal degeneration.[24] Furthermore, our result shows that the molecular layer was undulated in a wave-like appearance and it might look like be the same results of a finding in 2001.[25] we concluded that the use of LEV during pregnancy has a deleterious histological effect on the developing cerebral cortex of the newborn that should be carefully monitored.

REFERENCE

1. Swaroop HS, Ananya C, Nithin K, Jayashankar CA, Babu HS, Srinivas BN. Levetiracetam: A Review of its use in the treatment of epilepsy. IJMBR., 2013 Sep; 2(3): 166-72.
2. Krishna K, Raut AL, Gohel KH, Dave P. Drug Review. JAPI., 2011 Oct; 59: 653.
3. Tomson T, Palm R, Kallen k, Ben-Menachem E, Soderfeldt B, Danielsson B, et al. pharmacokinetics of levetiracetam during pregnancy, delivery, in the neonatal period, and lactation. Epilepsia., 2007 Jun; 48(6): 1111–16.

4. Radtke RA. Pharmacokinetics of Levetiracetam. Epilepsia., 2001 Aug; 42(S4): 24-27.
5. Omer H, Kutb M. Chronic histopathological effects of levetiracetam on some internal organs of adult albino rats. Egyptian Journal of Forensic Sciences., 2015; 5: 41-45.
6. Lynch BA, Lambeng N, Nocka K, Kensel-Hammes P, Bajjalieh SM, Matagne A, Fuks B. The synaptic vesicle protein SV2A is the binding site for the antiepileptic drug levetiracetam. Proceedings of the National academy of sciences of the United States of America., 2004 Jun 29; 101(26): 9861-6.
7. Shorvon SD, Van Rijckevorsel K. A new antiepileptic drug. J Neurol Neurosurg Psychiatry., 2002 Apr 1; 72(4): 426-29.
8. Yerby MS. Quality of life, epilepsy advances, and the evolving role of anticonvulsants in women with epilepsy. Neurology., 1999 Dec; 55(5 S1): S21-31.
9. Hvas CL, Henriksen TB, Østergaard JR, Dam M. Epilepsy and pregnancy: effect of antiepileptic drugs and lifestyle on birthweight. BJOG: An International Journal of Obstetrics & Gynaecology., 2000 Jul 1; 107(7): 896-902.
10. Pennell PB. Pregnancy in women who have epilepsy. Neurologic clinics., 2004 Nov 30; 22(4): 799-820.
11. Raedler A, Sievers J. The development of the visual system of the albino rat. Berlin: Springer Science & Business Media., 2012; 10.
12. Bolon B, Duryea D, Foley JF. Histo-technological processing of developing mice. In: Bolon B, editors. Pathology of the developing mouse: a systematic approach. Boca Raton: Taylor & Francis Group., 2015; 195-209.
13. Bancroft JD, Layton C. The hematoxylins and eosin. In: Theory and practice of histological techniques. Kim Suvarna S, Layton C, Bancroft JD, editor. 7th ed. Churchill Livingstone., 2013; 172-186.
14. Noback CR, Demarest RJ. The human nervous system basic principles of neurobiology. Chapter 4; Development and growth of the nervous system, Fong at Sons Printers Pte. Ltd. Singapore, 1984; 124-49.
15. Hernandez DS, Smith R, Wyszynski DP, Holmes LB. Malformations among infants exposed to Carbamazepine during pregnancy. Clin Mol Teratol., 2007; 79: 357.
16. Zahan CA. Neurologic care of pregnant women with epilepsy. Epilepsia., 1998; 39(8): S26-s31.
17. Kumar V, Abbas AK, Aster JC. Robbins and Cotran pathologic basis of disease. 9th ed. Philadelphia: Elsevier Saunders., 2015; 31-35.

18. Bittigau P, Sifringer M, Genz K, Reith E, Pospischil D, Govindarajalu S, Dzietko M, Pesditschek S, Mai I, Dikranian K, Olney JW. Antiepileptic drugs and apoptotic neurodegeneration in the developing brain. Proceedings of the National Academy of Sciences., 2002 Nov; 99(23): 15089-94.
19. Forcelli PA, Kim J, Kondratyev A, Gale K. Pattern of antiepileptic drug–induced cell death in limbic regions of the neonatal rat brain. Epilepsia., 2011 Dec 1; 52(12): e207-11.
20. Kim J, Kondratyev A, Gale K. Antiepileptic drug-induced neuronal cell death in the immature brain: effects of carbamazepine, topiramate, and levetiracetam as monotherapy versus polytherapy. Journal of Pharmacology and Experimental Therapeutics., 2007 Oct 1; 323(1): 165-73.
21. Mohan H. Textbook of pathology. 6th ed. New Delhi: Jaypee Brothers Medical Publishers., 2010; 105-07.
22. Hamdi H, El Ghareeb AEW, Kandil AM, Ahmed OM, Yahia R. The potential impacts of the anti-epileptic drug (oxcarbazepine) on albino rat's neonates during lactation. Asian Journal of Pharmaceutical and Clinical Research., 2016 Jul; 9(S1): 244-51.
23. Omer HA, Kutb MA. Chronic histopathological effects of levetiracetam on some internal organs of adult albino rats. Egyptian Journal of Forensic Sciences., 2015 Jun 30; 5(2): 41-5.
24. Hayashi H, Dikkes P, Soriano SG. Repeated administration of ketamine may lead to neuronal degeneration in the developing rat brain. Pediatric Anesthesia., 2002 Nov 1; 12(9): 770-4.
25. Graus-Porta D, Blaess S, Senften M, Littlewood-Evans A, Damsky C, Huang Z, Orban P, Klein R, Schittny JC, Müller U. β1-class integrins regulate the development of laminae and folia in the cerebral and cerebellar cortex. Neuron., 2001 Aug 16; 31(3): 367-79.