Egyptian Journal of Forensic Sciences (2015) 5, 41–45

HOSTED BY

Contents lists available at ScienceDirect

Egyptian Journal of Forensic Sciences

journal homepage: http://www.journals.elsevier.com/egyptian-journal-of-forensic-sciences

ORIGINAL ARTICLE

Chronic histopathological effects of levetiracetam on some internal organs of adult albino rats

Huda A.M. Omer [a,*], **Muna A.M. Kutb** [b]

[a] *Department of Forensic Medicine & Toxicology, Faculty of Medicine and Health Sciences, Aden University, Yemen*
[b] *Department of Histology, Faculty of Medicine and Health Sciences, Aden University, Yemen*

Received 20 April 2014; revised 18 May 2014; accepted 12 June 2014
Available online 15 August 2014

KEYWORDS

Levetiracetam;
Histopathology;
Liver;
Lung;
Focal necrosis

Abstract *Purpose:* To assess effects of levetiracetam (LEV) within its therapeutic range at a 54 mg/day and 1/4 LD50 = 70 mg/kg body weight for white albino male and female rats weighing an average of 180 ± 60 g has been studied in order to demonstrate whether LEV would affect the internal organs at the histological level.
Methods: Animals were randomly separated into control (n = 20), study group I (n = 20) and study group II (n = 20). They were obtained from the animal house, Assuit University. They were maintained in environmentally controlled rooms at a temperature of 28–32 °C, 40–60% humidity, in a noise free environment. Oral administration of 54 mg/day and 70 mg/kg LEV for groups I and II, respectively, was given while physiologic saline (0.045 ml) was given to the control group.
Results: Microscopic evaluation of the intestine, kidney, suprarenal glands and spleen, revealed that there was no statistical difference between the treated and control groups. Four specimens of the liver out of 20 (20%), showed focal necrosis around central veins. Lung sections that were obtained from 15 out of 20 (75.0%) rats in the study group II showed various histopathological findings compared to those of the control group. These findings include thickening of interstitial septa, interstitial fibrosis, chronic inflammatory infiltration of cells, and congestion of blood vessels.
Conclusions: LEV is considered as a safe drug in its therapeutic dose. Its safety needs further studies with long term follow-up.

Introduction

Levetiracetam (LEV) is a novel antiepileptic drug (AED) which was discovered in the early 1980s. In 1999 the FDA approved LEV monotherapy for the management of partial onset seizures. It has greatly increased the treatment options available to patients with generalized epilepsies[1] and refractory epilepsy.[2]

LEV {(S)-α-ethyl-2-oxo-pyrrolidine acetamide} is an analog of piracetam.[3] It is rapidly and completely absorbed after oral administration and it is predominantly eliminated as an unchanged drug in the urine. Its metabolism is independent of the cytochrome P450 enzyme system. LEV has not been

* Corresponding author. Tel.: +967 737581059.
E-mail addresses: hudafreedom@yahoo.com, kutb20@yahoo.com (H.A.M. Omer).
Peer review under responsibility of The International Association of Law and Forensic Sciences (IALFS).

http://dx.doi.org/10.1016/j.ejfs.2014.06.002

demonstrated to interact with other drugs in either direction.[4,5] Clearance of LEV is significantly reduced in patients with severe hepatic impairment and concomitant renal impairment (hepatorenal syndrome).[6]

LEV appears to act at the synaptic site by binding with the vesicle protein 2A (SV2A), and has a restraining effect on the secretion of neurotransmitters in the presynaptic area.[7,8] It inhibits secretion of calcium from neuronal stores and activation of neurons without interfering with normal activation. Additionally, it has been shown that LEV does not involve inhibitory and excitatory neuro-transmission.[9]

Materials and methods

A total number of sixty adult male and female albino rats weighing 180–220 g, were obtained from the animal house, Assuit University. They were maintained in environmentally controlled rooms at a temperature of 28–32 °C, 40–60% humidity, in a noise free environment and 12 h light–dark cycle. The female and male albino rats were kept in different spacious cages. All the rats had access to water and animal diet and libitum.

The animals were classified in three groups:

Control group: consisted of 20 (10 male and 10 female rats) with normal saline administration.

Group 1: consisted of 20 (10 male and 10 female rats) with therapeutic dose of LEV oral administration of 54 mg/day,[10] in two divided doses per day.

Group II: consisted of 20 (10 male and 10 female rats) with a high dose of LEV oral administration of $^{1}/_{4}$ LD_{50} = 70 mg/kg body weight,[11] in two divided doses per day.

LEV was given in its already prepared formulation (Tiratam®) an oral solution of 100 mg/ml from Al-Andalous for Pharmaceuticals-Egypt Industries. In all animals drugs were given orally by a gastric tube.

After sixty days of the experimental period each rat was killed by cervical dislocation, and the internal organs were obtained for histopathological study. Organs were preserved in 10% buffered neutral formalin. They were dehydrated and embedded in paraffin. Serial section 5 μm thick were cut and stained with hematoxylin and eosin (H and E). Observations were made by examining the serial section under light microscope.

Results

Microscopic examination, using H and E of LEV treated groups (therapeutic dose and $^{1}/_{4}$ LD_{50}) related histopathological changes in the intestine, kidney, spleen and suprarenal

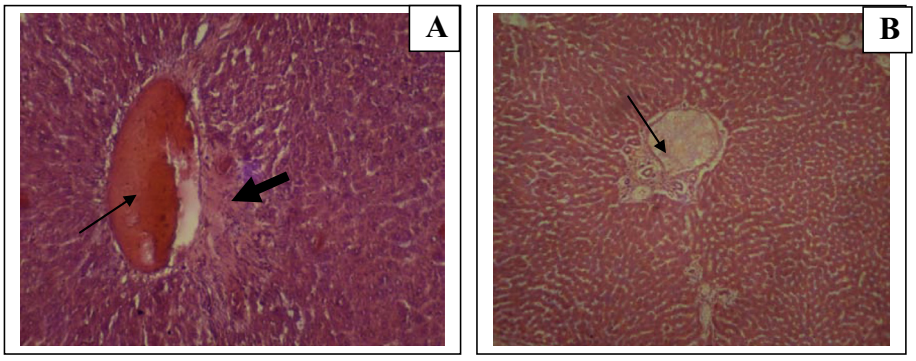

Figure 1 (A) Photomicrograph of the liver section in a animal treated with LEV in dose of $^{1}/_{4}$ LD_{50} showing central vein congestion (thin arrow), focal necrotic area (thick arrow) partial distortion of the liver architecture (H and E) (X200). (B) Photomicrograph of the liver section in a animal treated with LEV in dose of $^{1}/_{4}$ LD_{50} showing dilated congested portal vein (arrow) (H and E) (X100).

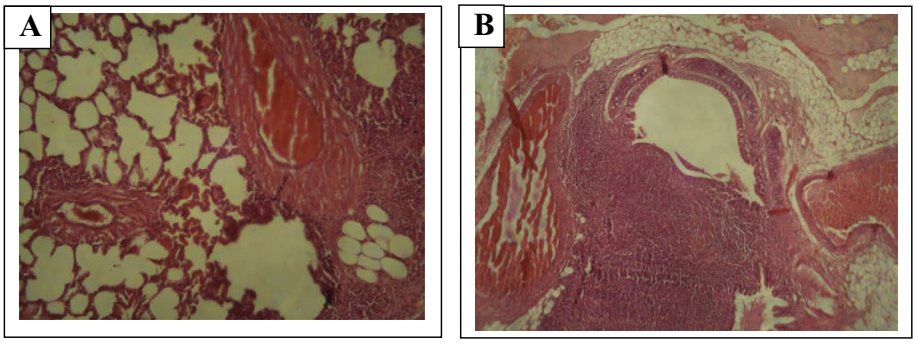

Figure 2 (A and B) Photomicrographs of lung sections in animals treated with LEV in a dose of $^{1}/_{4}$ LD_{50} showing vascular dilation and congestion with chronic inflammatory cell infiltration which destroyed the wall of a secondary bronchus. (A) (H and E) (X200), (B) (H and E) (X100).

glands revealing no significant difference in comparison with the control group.

No significant difference was found in the histological examination of liver sections in the tissue of animals treated with LEV 54 mg/day (Group I) as compared to those of controls while in the dose of 70 mg/day ($^1/_4$ LD_{50}) there were hepatic lesions. The most prominent microscopic changes consisted of centrilobular congestion in 70% and focal necrosis around the central vein 20% (Fig. 1A), dilated and congested portal veins 70% (Fig. 1B).

Light microscopic examination, using H and E, revealed that the study Group I exhibited no histopathological difference in comparison to the control group, while various histological findings were presented in lung sections obtained from the study group II (75.0%). These findings include: congestion of the blood vessels 85% (Fig. 2A and B) & (Fig. 3A, B and E). A thickened interalveolar (interstitial) septum and fibrosis was found in (90%) (Fig. 2A–D) and chronic inflammatory cell infiltration (90%), particularly around secondary bronchi and interstitial septa which may reduce the alveolar spaces for gaseous exchange (Figs. 2–4) (see Table 1).

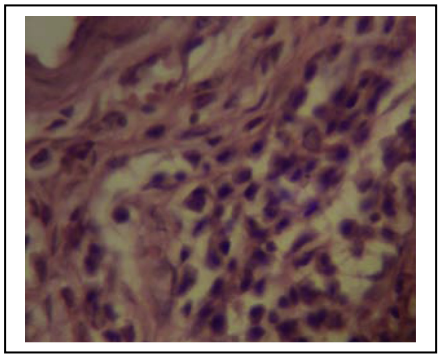

Figure 4 Photomicrograph of the lung section in a animal treated with LEV in a dose of $^1/_4$ LD_{50} showing chronic inflammatory cell infiltration (H and E) (X400).

Discussion

LEV-induced histopathological changes in internal organs (kidney, intestine, spleen and suprarenal glands) have not been reported. The present study evaluated the effect of LEV on the internal organs of the albino rats. Chronic administration of LEV at a therapeutic dose showed no histopathological differences as compared to those of control. Similar data are mentioned in a clinical study of Incecik et al., who suggest that the therapeutic dose of LEV does not alter liver functions.[12]

Our study showed that the hepatocellular and lung lesions occurred in those treated with a higher dose of LEV (group II).

Congestion of central veins, focal necrosis, and dilated and congested portal veins were detected in the liver sections of group II. Despite extensive uses of LEV there are no verified reports describing the possible histopathological side-effects, especially the hepatotoxic reactions. Overstreet et al. demonstrated that hepatocyte necrosis was found in those treated with lamotrigine.[13] Meshkibaf et al. mentioned that a higher dose of gabapentin caused scattered necrotic foci in liver

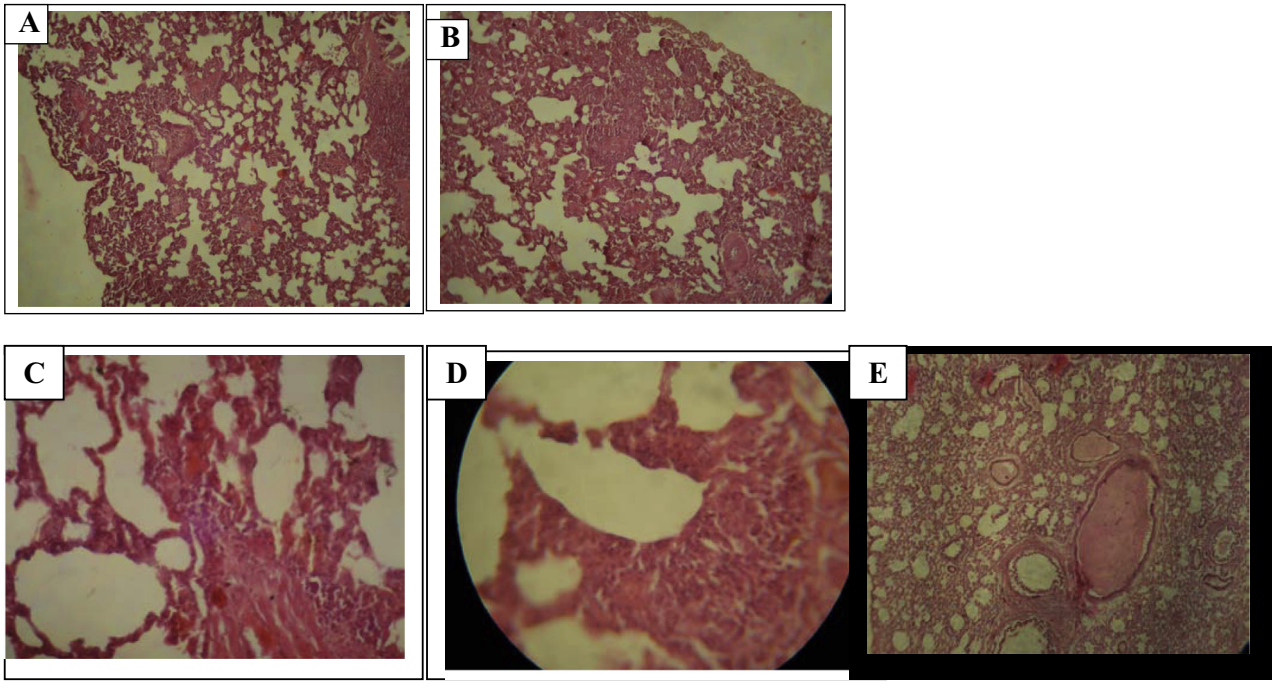

Figure 3 (A and B) Photomicrographs of lung sections in animals treated with LEV in a dose of $^1/_4$ LD_{50} showing vascular dilation and congestion with chronic inflammatory cell infiltration and thickening of interalveolar septum and interstitial fibrosis with obliteration of some alveolar spaces. (H and E) (X100). (C and D) Photomicrographs of lung sections in animals treated with LEV in a dose of $^1/_4$ LD_{50} showing high power of thickened alveolar septum (H and E) (X400). (E) Photomicrograph of the lung section in a animal treated with LEV in a dose of $^1/_4$ LD_{50} showing vascular dilation and congestion (H and E) (X100).

Table 1 Histopathological findings in a high-dose group II (20 animals).

Histopathological lesions	Severity of lesions											
	Mild (+)				Moderate (++)				Severe (+++)			
	Male		Female		Male		Female		Male		Female	
	No	%	No	%	No	%	No	%	No	%	No	%
Liver												
Centrilobular congestion (14 animals)	0	0.0	0	0.0	2	14.3	0	0.0	4	28.6	8	57.1
Focal necrosis around central vein (4 animals)	0	0.0	0	0.0	0	0.0	0	0.0	3	75.0	1	25.0
Dilated and congested portal veins (14 animals)	0	0.0	0	0.0	0	0.0	0	0.0	6	42.9	8	57.1
Lung												
Congestion of the blood vessels (17 animals)	0	0.0	0	0.0	1	5.9	2	11.8	9	52.9	5	29.4
Thickened interalveolar septum and fibrosis (18 animals)	0	0.0	0	0.0	0	0.0	0	0.0	11	61.1	7	38.9
Chronic inflammatory cell infiltration (18 animals)	0	0.0	0	0.0	0	0.0	0	0.0	9	50.0	9	50.0

parenchymal cells.[14] Saraswathy et al. reported that phenytoin-treated animals exhibited severe congestion, periportal inflammation, centrilobular congestion, fatty degeneration and hepatocellular necrosis.[15]

The mechanism of LEV-induced liver injury remains unknown. It is suggested that there may be underlying immunoallergic, genetic, toxic or acquired mitochondrial abnormalities as a major determinant to hepatotoxicity. Reactive metabolites from AED can in some cases, lead to direct cytotoxicity and liver cell necrosis, whereas in other cases this may lead to new antigen formation inducing immunoallergic mechanisms.[16,17]

The present results have demonstrated that a high dose of LEV causes histopathological changes in rat lungs. Congestion of the blood vessels, thickened interalveolar (interstitial) septum and fibrosis and chronic inflammatory cell infiltration were observed. These findings are in agreement with the study by Newsome et al.[16] who stated that LEV was implicated in the pathogenesis of interstitial pneumonitis suggesting that long term use of LEV could precipitate a diffuse interstitial pneumonitis-like reaction.[18] Saravanan et al. demonstrated that increased AED drug concentrations of lamotrigine triggered a diffuse interstitial process of relatively recent onset, with features consistent with diffuse lung disease.[19]

In agreement with our study is Travis et al. who reported that nonspecific interstitial pneumonia, lymphocytic interstitial pneumonia, or even patchy alveolar septal lymphoplasmacytic infiltrates without appreciable airway disease or parenchymal scarring are the most common pulmonary morphologic patterns that are associated with anticonvulsants and other agent toxicities.[20] Furthermore, Nikaido et al. and El Khayat et al. concluded that many antiepileptic drugs induced interstitial pneumonia and disturbed pulmonary functions for epileptic patients on regular prolonged use and they demonstrated that drug-induced interstitial lung disease should be considered as a possible complication of anticonvulsant treatment.[21,22]

The precise mechanism by which LEV exerts its lung injury with long-term use is unknown. Other anticonvulsant drugs such as carbamazepine appeared to be more injurious on lung parenchyma. A number of reports have highlighted pulmonary immune-mediated hypersensitivity to carbamazepine. Such hypersensitivity may involve type 3 immune complex and type 4 delayed hypersensitivity reaction.[23,24]

Funding

None.

Conflict of interest

None declared.

Ethical approval

Necessary ethical approval was obtained from the institute ethics committee.

References

1. Krishna K, Raut AL, Gohel KH, Dave P. Drug review: levetiracetam. *JAPI* 2011;**59**:52–4.
2. Gambardella A, Labate A, Quattrone A. Monotherapy for partial epilepsy: focus on levetiracetam. *Neuropsychiatr Dis Treat* 2008;**4**(1):33–8.
3. Dooley M, Plosker GL. Levetiracetam: a review of its adjunctive use in the management of partial onset seizures. *Drugs* 2000;**60**:871–93.
4. Shorvon SD. Pyrrolidone derivatives. *Lancet* 2001;**200**:1885–92.
5. Abou-Khalil BW. Does increased levetiracetam clearance during pregnancy require planned intervention? *Epilepsy Curr* 2008;**8**(3):62–3.
6. Lynch BA, Lambeng N, Nocka K, Kensel-Hammes P, Bajjalieh A, Matagne A, et al. The synaptic vesicle protein SV 2A is the binding site for the antiepileptic drug levetiracetam. *PNAS* 2004;**101**(26):9861–6.
7. Yang X-F, Weisenfeld A, Rothman SM. Prolonged exposure to levetiracetam reveals a presynaptic effect on neurotransmission. *Epilepsia* 2007;**48**:1861–9.
8. Pellock JM, Glauser TA, Bebin EM, Fountain NB, Ritter FJ, Coupez RM, et al. Pharmacokinetic study of levetiracetam in children. *Epilepsia* 2001;**42**:1574–9.
9. Somsak Tiamkao S, Thongplew S, Chawsamtong S, Sawanyawisuth K. Intravenous levetiracetam of hospitalized

patients in Srinagarind Hospital. *Afr J Pharm Pharmacol* 2013;**7**(18):1119–23.
10. Sugawara T, Kato M, Furuhama K, Inage R, Sazaki N, Takayame S. Single dose toxicity study of the new cognition-enhancing agent nefiracetam in mice, rat and doges. *Arzneimittelforschung* 1994;**44**(2A):211–3.
11. Paget GE, Barnes JM. Toxicological tests in evaluation of drug activities. In: Laurence DR, editor. *Pharmacometrics*. New York: Acad. Press; 1964. p. 134–5, 13.
12. Incecik F, Herguner MO, Altunbasak S. Levetiracetam and valproic acid: effects on the liver functions and ammonia level in children.. *Cukurova Med J* 2014;**39**(1):70–4.
13. Overstreet K, Costanza C, Behling C, Hassanin T, Masliah E. Fatal progressive hepatic necrosis associated with lamotrigine treatment: a case report and literature review. *Dig Dis Sci* 2002;**47**:1921–5.
14. Saraswathy GR, Maheswari E, Santhrani Thakur. Effect of vitamin C supplementation on phenytoin induced hepatotoxicity. *Global J Pharmacol* 2010;**4**(3):127–35.
15. Meshkibaf MH, Miladpoor B, Shole Var F, Abdollahi A. Chronic effect of gabapentin on liver function in adult male rat. *Acta Med Iran* 2013;**51**(12):830–3.
16. Newsome SD, Xue LY, Jennings T, Castaneda GY. Levetiracetam-induced diffuse interstitial lung disease. *J Child Neurol* 2007;**22**(5):628–30.
17. Travis WD, Colby TV, Koss MN, Rosado-de-Christenson ML, Muller NL, King TE. Drug and radiation reactions. In: King DW, editor. *Non-neoplastic disorders of the lower respiratory tract*. 1st ed. Washington, D.C.: American Registry of Pathology and the Armed Forces Institute of Pathology; 2002. p. 321–50.
18. El Khayat HA, Awadalla MM, Al Sharkawy AA, EL Khouly HA. Assessment of subtle pulmonary dysfunction in idiopaththic childhood epilepsy using impulse oscillometry. *EJB* 2012;**6**(2):95–103.
19. Saravanan N, Otaiku OM, Namushi RM. Interstitial pneumonitis during lamotrigine therapy. *Br J Clin Pharmacol* 2005;**60**(6):666–7.
20. Nikaido K, Kato T, Takayama R, Doi T. Valproate sodium and zonisamide associated interstitial pneumonitis in an infant. *No To Hattatsu* 2007;**39**(1):44–8.
21. Bjornsson E. Hepatotoxicity associated with antiepileptic drugs. *Acta Neurol Scand* 2008;**118**:281–90.
22. Santosa NAG, Medinaa WSG, Martinsa NM, Mingattob FE, Curtic C, Santos AC. Aromatic antiepileptic drugs and mitochondrial toxicity: effects on mitochondria isolated from rat liver. *Toxicol in Vitro* 2008;**22**:1143–52.
23. Mauri-Hellweg D, Bettens F, Mauri D, Brander C, Hunziker T, Pichler WJ. Activation of drug-specific CD4+ and CD8+ T cells in individuals allergic to sulfonamides, phenytoin, and carbamazepine. *J Immunol* 1995;**155**(1):462–72.
24. Wilschut FA, Cobben NA, Thunnissen FB, Lamers RJ, Wouters M, Drent M. Recurrent respiratory distress associated with carbamazepine overdose. *Eur Respir J* 1997;**10**(9):2163–5.