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Diclofenac sodium enhances the antiepileptic effect of levetiracetam in pilocarpine induced epileptic mice model

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Abstract: Epilepsy, a neuronal disorder has affected 1% of the world's population. Almost 35-40% of these patients get resistant to available anti-epileptic drugs (AEDs). Recent studies have shown the role of inflammation in the pathophysiology of epilepsy and a combination of anti-inflammatory and antiepileptic drugs could prove beneficial against epileptic seizures. Therefore, we aimed to examine the effect of levetiracetam (LEV) and diclofenac sodium (DFS) combination on pilocarpine (PLC) induced epileptic seizures in mice. Mice were divided into control and treatment groups. LEV alone and in combination with DFS was given for 3 days. On 3rd day after administering the required drugs, pilocarpine challenge was given intraperitoneally. Then, behavioral changes were observed for 90 minutes, including latency to first seizure, continuous seizures, duration of continuous seizures, and survival rate. Results showed significant improvement in the latencies to first ($P<0.001$) and continuous seizures ($P<0.05$), duration of the continuous seizure ($p=0.001$), and survival rate ($P<0.01$) in the combination treatment group as compared to the control or individual drug treatment groups. DFS enhances the efficacy of LEV, however, further mechanistic studies will be required to conclude if DFS can be given in combination with LEV for epilepsy treatment.

Keywords: Epilepsy, inflammation, pilocarpine, levetiracetam, diclofenac sodium.

INTRODUCTION

Epilepsy is a neurological disorder that has the tendency to generate recurrent seizures. Irrespective of the age group, epilepsy has affected around 50-70 million people worldwide (Trinka *et al*., 2019). Epileptic seizures are of two types; focal seizures- reported in 36% of epileptic patients and, generalized tonic-clonic seizures; mostly found in patients belonging to low- and middle-income states. Epilepsy is caused due to the hyper-excitability of neurons resulting in the excessive discharge of neurotransmitters (Chou *et al*., 2020).

Currently available anti-epileptic drugs (AEDS) effectively reduce the neuronal responses of the brain by averting the neuronal depolarization either via obstructing calcium or sodium channels, decreasing the excitation caused by the neurotransmitter glutamate, increasing the function of potassium channels, or enhancing the inhibitory effect of GABA (Glauser *et al*., 2013). Despite the availability of these drugs approximately 30-40% of epileptic patients develop resistance and do not respond to the available therapies (Yang *et al*., 2021). Therefore, there is a dire need to identify drugs that can be used in combination with available AEDs to enhance their efficacy and to target other etio-pathological factors such as inflammation.

The role of inflammation in epileptogenesis has been revealed through extensive experimental studies on animal models of epilepsy and resected human brain tissues of epileptic patients. Studies have reported elevated expression of inflammatory markers like 1β, IL-6, and TNF-α in epileptic animal models and patients. Ravizza *et al*. reported increased levels of IL-1β in astrocytes and microglial cells during epileptic seizures (Ravizza and Vezzani, 2006). Sinha and his colleagues found increased levels of proinflammatory cytokines in the serum of epilepsy patients in their post-ictal phase (Sinha *et al*., 2008). Studies have also reported elevated levels of IL-6 in the cerebrospinal fluid of the epileptic patients with generalized tonic-clonic seizures as compared to their plasma levels (Webster *et al*., 2017, Alapirtti *et al*., 2018) and it was also found increased in the serum during post-ictal phase (Gruol, 2015).

Therefore, it is suspected that a potential anti-inflammatory molecule could curtail epileptic seizures. For the same reason, some studies have evaluated the role of non-steroidal anti-inflammatory drugs. Further, When Jung *et al*, treated pilocarpine-induced status epilepticus with celecoxib (COX-2 inhibitor) the neuronal loss and

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microglial activation were prevented in the hippocampus of rodents (Jung *et al*., 2006). In another study when celecoxib was combined with prostaglandin E2 (PGE2) antibodies the seizures were reduced in pentylenetetrazol (PTZ) models (Oliveira *et al*., 2008). Diclofenac Sodium (DFS) is a non-steroidal anti-inflammatory drug (NSAIDs) drug, which is beneficial because of its inhibition of prostaglandin (PG) at the site of inflammation (Altman *et al*., 2015). It is a potent cyclooxygenase inhibitor that possesses anti-inflammatory, antipyretic, and analgesic properties. It acts selectively on COX-2 but also acts non-selectively on COX-1 (Ajmone-Cat *et al*., 2010).

The improvement in epileptic seizures due to the use of NSAIDS suggests that they could be used in combination with AEDS and may become a possible therapy for epileptic patients who are resistant to available AEDS. Since no data is available regarding the combination of DFS and standard AED i.e. Levetiracetam (LEV) against epilepsy, the present study is therefore planned to evaluate the effect of DFS in combination with levetiracetam (LEV) on the severity of seizures in pilocarpine (PLC) induced epilepsy mice model.

MATERIALS AND METHODS

Animals

Albino male Naval Medical Research Institute (NMRI) strain mice weighed around 24-30grams were used in the study. The mice were given free access to food and water and kept in 12 hours light and dark cycle. All the experimental procedures were performed in accordance with the ethical guidelines defined by Institutional Review Board and Animal Ethical Committee DUHS. Approval IDs are IRB-1332/DUHS/Approval/2019/ and AR.IRB-015/DUHS/Approval/2019/025 respectively. International guidelines for animal use and care by Committee on Animal Research and Ethics (CARE) were also followed.

Drugs Dosage

All clinical standard drugs were purchased from local pharmacies. Dilutions were made by utilizing commercially available sterile normal saline. Doses (mg) were calculated for administration according to the weight of animals, followed by the volume to be administered (mg/ml). For DFS, 2.5mg/ml drug solution was prepared and only 0.1ml of this solution was used to be administered intraperitoneally to provide a 10mg/kg dose. (Hasani *et al*., 2011, Lahoti *et al*., 2014). For LEV, 15mg/kg dose was prepared by making a dilution with 5 mg/ml strength and a final volume of 0.08 ml of this solution was injected *i.p*. to fulfill dose requirements. (Loscher and Honack, 1993; Klitgaard *et al.*, 1998; McArthur and Borsini, 2008). For PLC: a 200mg/kg dose was prepared by using a 2% solution with a concentration of 20mg/ml and finally, 0.25 ml of this solution was given subcutaneously to mice. (Curia *et al.,* 2008; Vizzani, 2009)

Experimental Groups

Animals were divided into the following five experimental groups (n=12/group).

(i) Normal control (received 0.9% normal saline), (ii) Disease model group i.e. PLC only, (administered 200mg/kg pilocarpine), (iii) drugs only control i.e. LEV + DFS (The animals were administered 10mg/kg diclofenac sodium and 15mg/kg levetiracetam with a difference of 15 minutes). (iv) LEV only treatment group i.e. LEV + PLC group (received 15mg/kg levetiracetam and 200mg/kg pilocarpine) and (v) drug combination treatment group i.e. LEV+ DFS + PLC administered 10mg/kg diclofenac sodium and after 15mins, 15mg/kg Levetiracetam and 200mg/kg pilocarpine).

The drugs were administered intraperitoneally for 3 consecutive days. On the third day, a challenge dose of pilocarpine (200mg/kg) was given to the mice in all the experimental groups except the normal control and the drug combination control group. Following the administration, the behavioral changes of the mice were observed.

Behavioural Evaluation

After administration of the pilocarpine challenge dose, behavioural changes were evaluated for 90 minutes. The parameters evaluated included survival percentage, latencies to first and continuous seizures, duration of continuous seizures, and the percentage of mice that experienced continuous seizures.

Survival percentage

After the administration of pilocarpine, levetiracetam, and diclofenac sodium to all the mice according to their respective groups, they were observed for 90 minutes. The number of mice that experienced extreme status epilepticus leading to death and the number of mice that survived were counted and used to calculate the survival percentage of the total mice population.

Latency to first and continuous seizures

After the drugs administration the mice were examined to observe the onset of first and continuous seizures. The time delay in which they experienced seizures was selected as their latency to first and continuous seizures.

Duration of continuous seizures

The mice were observed from the start of continuous seizures till the time they were dissected. This period was calculated as the duration of continuous seizures.

STATISTICAL ANALYSIS

The data were analyzed using a statistical package for the social sciences (SPSS)-21 software. Further, the data were

analyzed using one-way analysis of variance (ANOVA) followed by Bonferroni's post hoc test, and all the p values below 0.05 were considered significant.

RESULTS

Behavioural Evaluations

After the administration of pilocarpine, diclofenac sodium, and levetiracetam according to their respective experimental groups, the mice were evaluated for 90mins for the following parameters.

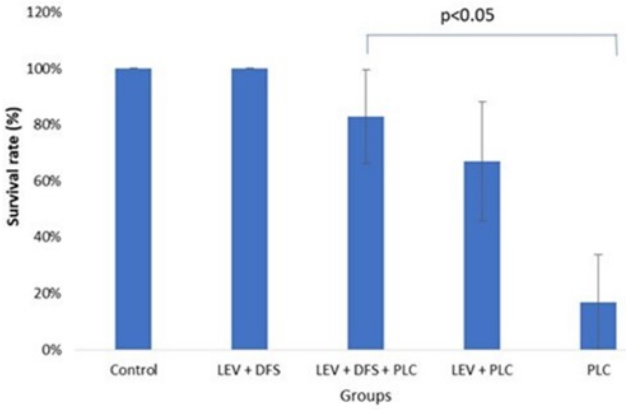

Fig. 1: Effect of drug combination on the percentage of survival rate of mice. The percentage survival was observed 100% in the control and LEV + DFS groups. The least survival rates were observed in the PLC group. While the LEV+DFS+PLC group has a higher survival rate when compared with the LEV+PLC group. A significant increase in the survival rate was observed LEV+DFS+PLC in comparison PLC group.

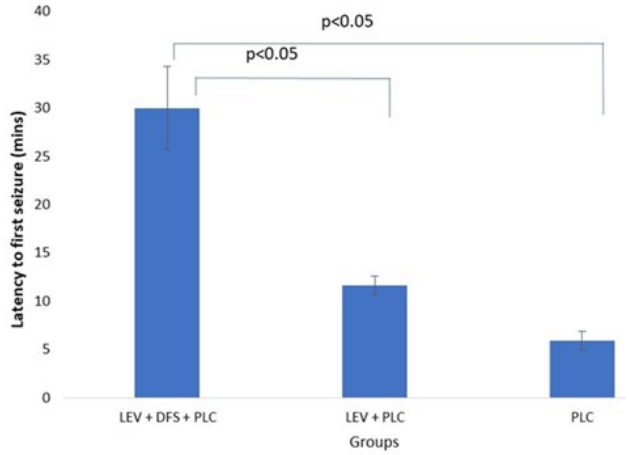

Fig. 2: Effect of drug combination on mean latency to first seizure. The longest delay to the first seizure was observed in the LEV+DFS+PLC group of about 30mins, while when compared with the LEV+PLC group they experienced it within 12mins, a statistically significant increase in LEV + DFS + PLC group. The least latency was observed in the PLC group of about 6mins when compared with LEV + DFS + PLC group there was a significant decrease in PLC alone.

Effect of drugs on survival rate

When the experimental groups were evaluated for the effect of drugs on survival rate, it was observed to be 100% in the normal control and drugs only control group i.e. LEV + DFS (fig. 1) On the contrary, the survival rate in the disease model group (PLC only) was reduced to 17%. In comparison to PLC, only the survival was much higher (58%) in animals receiving LEV treatment (LEV + PLC) which was further improved to 85% in the combination treatment group (LEV+DFS+PLC), ($P \leq 0.05$).

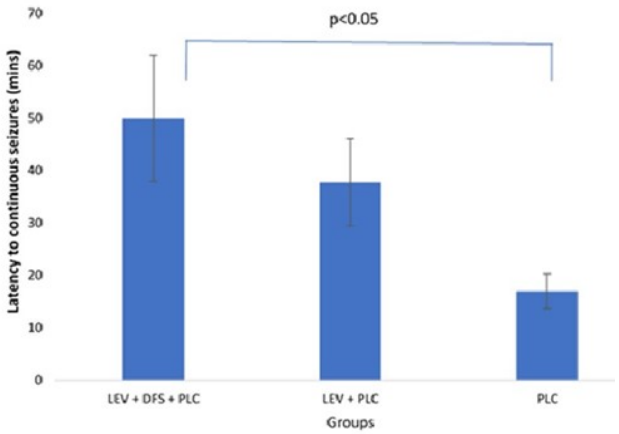

Fig. 3: Effect of drug combination on mean latency to continuous seizures. The latency for the continuous seizures was significantly increased to approximately 50 mins in the LEV+DFS+PLC group from 17mins as compared to the PLC group. While the LEV+PLC group experienced continuous seizures within 38mins.

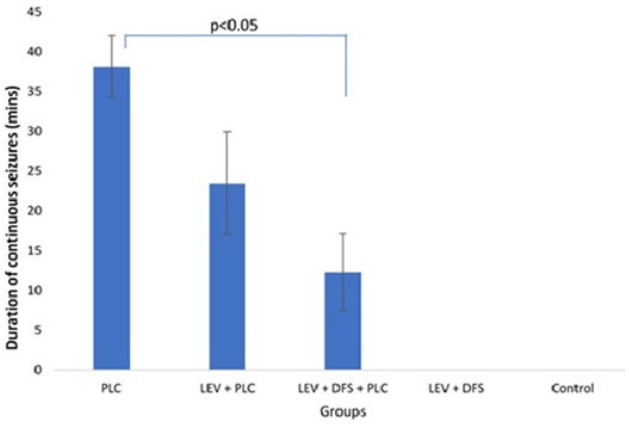

Fig. 4: Effect of drug combination on the duration of continuous seizures. The longest duration of continuous seizures was observed in the PLC group while no continuous seizures were observed in the control and LEV+DFS group. In the LEV+DFS+PLC group, the duration of continuous seizures was 12mins which when compared to the PLC group there was a significant increase to 38mins. In the LEV+PLC group, the continuous seizures remained for 24mins.

Latencies to First Seizure

When the pilocarpine challenge was given to the mice, it was observed that the seizures started in the first 30-40mins. The mice experienced different types of seizures, some experienced mild to severe head and neck seizures at the start while in some mice the first seizure was a full-body jerk. The shortest delay in the first seizure was around 6mins and was observed in the disease model i.e. PLC only group and they had seizure onset with a full-body jerk. Whereas the mice of the combination treatment group experienced the longest delay of about 30 minutes. This increase in latency to first seizure was significant as compared to either PLC only or the LEV+PLC group ($P< 0.001$). The control groups experienced no epileptic seizures hence ignored in the fig. 2.

Latency to Continuous Seizures

When the mice were evaluated for latency to continuous seizure, the disease model group experienced the onset of severe continuous seizures within 17 minutes and their severity was observed to be increasing with time. In the case of the LEV+PLC group, the latency to continuous seizures was around 38 mins. However, the difference in latency was not significant when compared to PLC only group ($P>0.05$). While a significant increase in the latency to continuous seizures (50 mins) was observed in the combination treatment group i.e. LEV+DFS+PLC as compared to the disease model or PLC only group ($P<0.05$). The control group and the combination group had no continuous seizures therefore they were not included in fig. 3.

Duration of Continuous Seizures

The next parameter evaluated was the average duration of continuous seizures (fig. 4). The continuous seizures experienced by the disease model group were of increased severity leading to death and longest duration i.e. 38mins. When the disease group was compared with the combination treatment group (DFS+LEV+PLC) not only there was a significant decrease in the duration of continuous seizures but there was a difference in the severity of the epileptic seizures also ($P<0.001$). While no significant difference was observed when PLC was only compared with the LEV+PLC group. The control and the combination group had no seizures at all during the 90mins of behavioral evaluation after the drug administration.

DISCUSSION

Regardless of the advances in the research on epilepsy, countless patients have recurrent seizures even while undergoing treatments and among them, around 30-40% are resistant to the available AEDS (Yang *et al*., 2021). Therefore, there is a need to elucidate the pathophysiological mechanisms of epilepsy to acquire new therapies. Inflammation is one of the pathophysiological mechanisms causing epilepsy. Several experimental studies have reported the role of inflammation in epileptogenesis as it is increasing the proinflammatory cytokines levels resulting in neuronal damage (Billiau *et al*., 2007, Sinha *et al*., 2008, Gruol and D. L., 2015, Li *et al*., 2018). In this regard, we aimed to investigate the combined protective effect of Levetiracetam and Diclofenac sodium on PLC induced epileptic seizures in different experimental groups.

The results of our study demonstrated that there was a significant increase in the survival rate of treatment groups which was maximum in the combination treatment group, showing the neuroprotective effect of Levetiracetam which was further enhanced with diclofenac. Whereas the lowest survival rates in diseases models were declined due to the proconvulsant nature of pilocarpine and excessive activation of muscarinic receptors. These results were in-lined with the study of Oliveria *et al* 2005, providing evidence that pilocarpine induces high mortality rates while it is decreased in the mice treated with levetiracetam (Oliveira *et al*., 2005).

The delay in the mean latencies in the onset of first and continuous seizures observed in our study was similar to the results found in several studies 16-17 (Glien *et al*., 2002, Zheng *et al*., 2010). The latency was decreased by LEV via inhibition of calcium influx which leads to reduced neurotransmitter release 18(Klitgaard, 2001) and prolonged hyperpolarization of GABA gated current 19 (Niespodziany *et al*., 2001). On the other hand, the duration of the seizures was significantly decreased in the combination treatment group. This was possible because LEV targeted the synaptic vesicle protein 2A (SV2A) preventing neurotransmitters' release 20-21 (Rigo *et al*., 2002, Nowack *et al*., 2011), and DFS worked as a cyclooxygenases inhibitor preventing inflammation which is a well-known pathophysiological factor in epileptogenesis (Vezzani *et al*., 2000, Lynch *et al*., 2004, Maroso *et al*., 2011a, Maroso *et al*., 2011b). The data revealed that there was a synergistic effect of diclofenac sodium on the efficacy of levetiracetam. These results are further supported by the study of Almaghour *et al* in which the duration of seizures was reduced in mice by the combined use of different NSAIDs with another antiepileptic drug i.e. diazepam (Almaghour *et al*., 2014).

As LEV and DFS is a novel drug combination with an unknown pathway of their combined effect, it was possible that the combination would have led to some severe reactions. For this reason, the combination group (LEV + DFS) was investigated in which no mice suffered seizures at all and when they were dissected their organs showed normal morphological features as observed in the control group.

Further, the present study is study limited to the acute model, and only behavioral aspect has been taken into

consideration, in future molecular studies on the same drug combination and on chronic epileptic models should be done.

CONCLUSION

From the results of the present study, it can be concluded that diclofenac sodium increases the efficacy of levetiracetam against epileptic seizures. However, detailed new molecular and mechanistic studies are needed to further confirm their combined utility in epileptic patients.

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REFFERENCES

Ajmone-Cat MA, Bernardo A, Greco A and Minghetti L (2010). Non-steroidal anti-inflammatory drugs and brain inflammation: effects on microglial functions. *Pharmaceuticals,* **3**: 1949-1965.

Alapirtti T, Lehtimaki K, Nieminen R, Makinen R, Raitanen J, Moilanen E, Makinen J and Peltola J (2018). The production of IL-6 in acute epileptic seizure: a video-eeg Study. *J. Neuroimmunol.,* **316**: 50-55.

Almaghour H, Zawawi N and Sherif F (2014). Effects of non-steroidal anti-inflammatory drugs on anti-convulsant activity of diazepam in mice. *Pharm Pharmacol. Int. J.,* **1**(1): 118-124.

Altman R, Bosch B, Brune K, Patrignani P and Young C (2015). Advances in nsaid development: evolution of diclofenac products using pharmaceutical technology. *Drugs,* **75**(8): 859-877.

Billiau AD, Witters P, Ceulemans B, Kasran A, Wouters C and Lagae L (2007). Intravenous immunoglobulins. *In*: Refractory childhood-onset epilepsy: Effects on seizure frequency, eeg activity and cerebrospinal fluid cytokine profile. *Epilepsia,* **48**(9): 1739-1749.

Chou P, Wang GH, Hsueh SW, Yang YC and Kuo CC, (2020). Delta-frequency augmentation and synchroni-zation. *In*: Seizure discharges and telencephalic transmission. *iScience,* **23**(11): 101666.

Curia G, Longo D, Biagini G, Jones RS and Avoli M, (2008). The pilocarpine model of temporal lobe epilepsy. *J. Neurosci. Methods.,* **172**(2): 143-157.

Glauser T, Ben-Menachem E, Bourgeois B, Cnaan A, Guerreiro C, Kalviainen R, Mattson R, French JA, Perucca E and Tomson T (2013). Updated ilae evidence review of antiepileptic drug efficacy and effectiveness as initial monotherapy for epileptic seizures and syndromes. *Epilepsia,* **54**(3): 551-563.

Glien M, Brandt C, Potschka H and Loscher W (2002). Effects of the novel antiepileptic drug levetiracetam on spontaneous recurrent seizures in the rat pilocarpine model of temporal lobe epilepsy. *Epilepsia,* **43**(4): 350-357.

Gruol DL (2015). IL-6 regulation of synaptic function in the CNS. *Neuropharmacology,* **96**: 42-54.

Hasani AS, Soljakova M, Jakupi MH and Ustalar-Ozgen SZ (2011). Preemptive analgesic effect of diclofenac: Experimental study in rats. *Middle East J. Anesthesiol.,* **21**(3): 355-360.

Jung KH, Chu K, Lee ST, Kim J, Sinn DI, Kim JM, Park DK., Lee JJ, Kim SU and Kim M (2006). Cyclooxygenase-2 inhibitor, celecoxib, inhibits the altered hippocampal neurogenesis with attenuation of spontaneous recurrent seizures following pilocarpine-induced status epilepticus. *Neurobiol. Dis.,* **23**(2): 237-246.

Klitgaard H (2001). Levetiracetam: The preclinical profile of a new class of antiepileptic drugs? *Epilepsia,* **42**: 13-18.

Klitgaard H, Matagne A, Gobert J and Wülfert E (1998). Evidence for a unique profile of levetiracetam in rodent models of seizures and epilepsy. *Eur. J. Pharmacol.,* **353**(2-3): 191-206.

Lahoti A, Kalra BS and Tekur U (2014). Evaluation of the analgesic and anti-inflammatory activity of fixed dose combination: Non-steroidal anti-inflammatory drugs in: experimental animals. *Indian J. Dent. Res.,* **25**(5): 551.

Li TR, Jia YJ, Ma C, Qiu WY, Wang Q, Shao XQ and Lv RJ (2018). The role of the microrna-146a/complement factor h/interleukin-1β-mediated inflammatory loop circuit. *In*: the perpetuate inflammation of chronic temporal lobe epilepsy. *Dis Model Mech.,* **11**(3): P.Dmm031708.

Loscher W and Honack D (1993). Profile of Ucb L059, a novel anticonvulsant drug, in models of partial and generalized epilepsy in mice and rats. *Eur. J. Pharmacol.,* **232**(2-3):147-158.

Lynch BA, Lambeng N, Nocka K, Kensel-Hammes P, Bajjalieh SM, Matagne A and Fuk B (2004). The synaptic vesicle protein sv2a is the binding site for the antiepileptic drug levetiracetam. *Proc. Natl. Acad. Sci. USA.,* **101**(26): 9861-9866.

Maroso M, Balosso S, Ravizza T, Iori V, Wright CI, French J and Vezzani A (2011a). Interleukin-1β biosynthesis inhibition reduces acute seizures and drug resistant chronic epileptic activity in mice. *Neurotherapeutics,* **8**(2): 304-315.

Maroso M, Balosso S, Ravizza T, Liu J, Bianchi M and Vezzani A (2011b). Interleukin-1 Type 1 Receptor/Toll-Like Receptor Signalling In Epilepsy: The importance of il-1beta and high-mobility group Box 1. *J. Intern. Med.,* **270**(4): 319-326.

Mcarthur RA and Borsini F (Eds.) (2008). Animal and translational models for CNS drug discovery: Neurological Disorders. Academic Press.

Niespodziany I, Klitgaard H and Margineanu DG (2001). Levetiracetam inhibits the high-voltage-activated ca2+ current in pyramidal neurones of rat hippocampal slices. *Neuroscience Letters,* **306**(1-2): 5-8.

Nowack A, Malarkey EB, Yao J, Bleckert A, Hill J and Bajjalieh SM (2011). Levetiracetam reverses synaptic deficits produced by overexpression of Sv2a. *Plos One,* **6**(12): E29560.

Oliveira A, Nogueira C, Nascimento V, Aguiar L, Freitas R, Sousa F, Viana G and Fonteles M (2005). Evaluation of levetiracetam effects on pilocarpine-induced seizures: cholinergic muscarinic system involvement. *Neuroscience Letters,* **385**(3): 184-188.

Oliveira M, Furian A, Rambo L, Ribeiro L, Royes L, Ferreira J, Calixto J and Mello C (2008). Modulation of pentylenetetrazol-induced seizures by prostaglandin E2 receptors. *Neuroscience,* **152**(4): 1110-1118.

Ravizza T and Vezzani A (2006). Status epilepticus induces time-dependent neuronal and astrocytic expression of interleukin-1 receptor type I in the rat limbic system. *Neuroscience,* **137**(1): 301-308.

Rigo JM, Hans G, Nguyen L, Rocher V, Belachew S, Malgrange B, Leprince P, Moonen G, Selak I and Matagne A (2002). The anti-epileptic drug levetiracetam reverses the inhibition by negative allosteric modulators of neuronal gaba-and glycine-gated currents. *Br. J. Pharmacol.,* **136**(5): 659-672.

Sinha S, Patil S, Jayalekshmy V and Satishchandra P (2008). Do cytokines have any role in epilepsy? *Epilepsy Research,* **82**(2-3): 171-176.

Trinka E, Kwan P, Lee B and Dash A (2019). Epilepsy in Asia: Disease burden, management barriers and challenges. *Epilepsia,* **60**: 7-21.

Vezzani A (2009). Pilocarpine-Induced Seizures revisited: What does the model mimic? *Epilepsy Currents*, **9**(5): 146-148.

Vezzani A, Moneta D, Conti M, Richichi C, Ravizza T, De Luigi A, De Simoni M, Sperk G, Andell-Jonsson S and Lundkvist J (2000). Powerful anticonvulsant action of il-1 receptor antagonist on intracerebral injection and astrocytic over expression in mice. Proc. Natl. Acad. Sci. **97**(21): 11534-11539.

Webster KM, Sun M, Crack P, O'brien TJ, Shultz SR and Semple BD (2017). Inflammation in epileptogenesis after traumatic brain injury, *J Neuroimmunol.,* **14**(1): 1-17.

Yang S, Wang B and Han X (2021). Models for predicting treatment efficacy of antiepileptic drugs and prognosis of treatment withdrawal. *In*: Epilepsy patients. *Acta Epileptologica,* **3**(1): 1-6.

Zheng Y, Moussally J, Cash SS, Karnam HB and Cole AJ (2010). Intravenous levetiracetam in the rat pilocarpine-induced status epilepticus model: Behavioral, physiological and histological studies. *Neuropharmacology,* **58**(4-5): 793-798.