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Research Article

Diclofenac Influence on the Anticonvulsant Effect of Retigabine: The Potential Role of KCNQ Channels

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Abstract. Retigabine is a new antiepileptic drug. Its mechanism of action involves activation of voltage gated potassium channel (Kv7, KCNQ). Epilepsy is a disease of chronic nature and may be associated with other diseases that need therapy with other drugs as NSAIDs like diclofenac. *Purpose.* The current work is designed to study the spectrum of retigabine and diclofenac, on acute seizures in albino mice; also, it investigated the possible interactions between retigabine and diclofenac regarding their anticonvulsant activities and the role of KCNQ channel. *Method.* Convulsions were induced in mice using the picrotoxin model, the pilocarpine-induced-status epilepticus model, and lastly the maximal electroshock model. *Results.* The study revealed that combined administration of retigabine and diclofenac significantly increased percentage of protection from convulsions induced by picrotoxin model and MES test. Furthermore, the combination significantly prolonged the mean latency period of convulsion in pilocarpine-induced sustained epilepsy model. *Conclusion.* we concluded that retigabine has a broad spectrum antiepileptic effect and diclofenac potentiated retigabine action as an opener of potassium (KCNQ2/3) channels.

Keywords: epilepsy; retigabine; diclofenac; pilocarpine; picrotoxin; 4-aminopyridine.

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1. Introduction

Epilepsy is a neurological disorder characterized by repeated seizure, caused by excessive discharge of neurons that affect 0.5-1% of population [9]. Traditional antiepileptic drugs have effect on 60-70% of the population with epilepsy. Other patients with epilepsy do not respond well to the classical antiepileptic drugs [7].

Retigabine is a new antiepileptic drug approved by the European Medicines Agency in January 2011. Its mechanism of action involves neuronal hyperpolarization and stabilization of the resting membrane potential via activation of voltage gated potassium channel (Kv7, KCNQ) [13].

Sills et al. (2000) showed that retigabine has been found to increase GABA synthesis in rat brain and enhance the chloride current. It also showed a decrease in the concentrations of glutamine and glutamate in rat brain, which may be another mechanism of action.

KCNQ (Kv7) channels are a subtype of voltage gated k channels. Kv7 channels play an important role in the regulation of neuronal action potential initiation and propagation, and so they have a major effect on brain excitability [21].

Epilepsy, as a disease of chronic nature, may be associated with other diseases, as rheumatoid arthritis and low back pain syndrome, that need therapy with other drugs as NSAIDs like diclofenac.

Interestingly, diclofenac may be one of the novel k channel openers, activating the KCNQ2\Q3 channels [18], and this new mechanism of action may help in epilepsy. Through the action of diclofenac on this channel, the action of retigabine can be affected.

The current work aimed at studying the spectrum of retigabine and diclofenac, on maximal electric shock (MES) induced hind limb extension, pilocarpine, and picrotoxin-induced acute seizures in albino mice. The present study also investigated the possible interactions between retigabine and diclofenac regarding their anticonvulsant activities.

2. Materials and Methods

2.1. Animals

The study was done on adult male albino mice, 8 weeks old, weighing 22-26 g, purchased from the Faculty of Veterinary Medicine, Zagazig University, Egypt. The animals were kept under standard laboratory conditions (temperature of 22 ± 1∘C, natural light-dark cycle), with free access to standard diet and tap water, housed in metal cages. After 7 days of adaptation to standardized housing conditions, the animals were randomly assigned to experimental groups. Each mouse was used only once, and all tests were performed between 8.00 and 15.00h. All experimental protocols were approved by the Ethics Committee of Zagazig University. This work has been carried out in accordance with the "Guide to the Care and Use of Experimental Animal Care".

All efforts were made to minimize the number of animals used and their suffering.

2.2. Drugs

- *Retigabine*, powder, was purchased from BOC SCIENCES, USA.

- *Picrotoxin*, powder, was purchased from Sigma Co., Egypt.

- *Diclofenac*, powder, was purchased from sigma Co., Egypt.

- *Lithium chloride*, powder, was purchased from sigma Co., Egypt.

- *Pilocarpine hydrochloride*, powder, was purchased from sigma Co., Egypt.

- *4-Aminopyridine* was purchased from sigma Co., Egypt.

2.2.1. Induction of convulsion by picrotoxin

Picrotoxin (5 mg/kg) was administrated intraperitoneally (i.p.) to induce seizures [19].

The following parameters were recorded: latency period of seizure in minutes, percentage of convulsion, and percentage of protection from convulsions and death rate.

Experimental groups

a- Control group: 6 mice were injected with picrotoxin 5mg/kg/i.p.

b- Retigabine group: animals were injected with retigabine i.p. 15 minutes later, they received picrotoxin 5 mg/kg/i.p., divided into 4 subgroups: R1, R2, R3, and R4, with each group consisting of 6 animals injected with retigabine (2, 4, 8, and 12mg/kg) and later picrotoxin (5mg/kg).

Retigabine (4mg/kg) was the medium dose, so it was selected for interaction.

c- Diclofenac group: animals were injected with diclofenac i.p. 1hr later, they received picrotoxin 5 mg/kg/i.p., divided into 3 subgroups: D1, D2, and D3, with each group consisting of 6 animals injected with diclofenac (5, 10, and 20mg/kg) and later picrotoxin (5mg/kg).

Diclofenac (10mg/kg) was the medium dose, so it was selected for interaction.

d- Combined group: 6 animals received diclofenac 10mg/kg/i.p. 45 minutes later, they received retigabine at 4 mg/kg, i.p. 15 minutes later, they received picrotoxin 5 mg/kg/i.p.

2.2.2. Pilocarpine-induced sustained epilepsy (SE)

- Lithium chloride 127.17 mg/kg/ i.p. was injected 24 hours before pilocarpine.

- Pilocarpine hydrochloride (350 mg/kg/ i.p.) was used to induce SE according to Jung et al. (2007).

  – *The following parameters were recorded:* latency period to first seizures in minutes, percentage of convulsion, and percentage of protection from convulsions and death rate.

Experimental groups

a- Control group: 6 mice were injected with pilocarpine 350 mg/kg/i.p.

b- Retigabine group: animals were injected with retigabine i.p. 15 minutes later, they received pilocarpine 350 mg/kg/i.p. and were subdivided into R1, R2, R3, and R4, with each group consisting of 6 animals injected with retigabine (2, 4, 8, and 12mg/kg/i.p.).

Retigabine (8mg/kg) was the medium dose, so it was selected for interaction.

c- Diclofenac group: animals were injected with diclofenac. 1hr later, they received pilocarpine 350 mg/kg/i.p. and were subdivided into 3 subgroups: D1, D2, and D3, with each group consisting of 6 animals injected with diclofenac (5, 10 and 20 mg/kg/i.p.).

Diclofenac (10mg/kg) was the medium dose, so it was selected for interaction.

d- Combined group: 6 animals were injected with diclofenac 10mg/kg/i.p. 45 minutes later, they were injected with retigabine at 8 mg/kg/i.p. 15 minutes later, they received pilocarpine 350 mg/kg.

2.2.3. Maximal electroshock seizure threshold (MEST) test

Apparatus: a Rodent Shocker generator was used (constant-current stimulator Type 221, Hugo Sachs Elektronik, Freiburg, Germany) (Figure 1).

Figure 1

Procedure: Electroconvulsions (25 mA, 50 Hz, 500 V, 0.2 s stimulus duration) , and delivered via standard [31] auricular electrodes. The endpoint was the tonic extension of the mouse hind limbs.

The following parameters were recorded: duration of hind limb extension, percentage of convulsion, and percentage of protection from convulsions and death rate.

In this model we use 4- Aminopyridine as voltage gated k channel blocker (K_v blocker).

Experimental groups

a- Control group: 6 animals were subjected to electric-shock of fixed current intensity 25mA for 0.2 s duration.

b- Retigabine group: animals were injected with retigabine 15 min before MES test, divided into 4 subgroups: R1, R2, R3, and R4, with each group consisting of 6 animals injected with retigabine (2, 4, 8, and 12mg/kg).

- Retigabine (8mg/kg) was the medium dose, so it was selected for interaction.

c- Diclofenac group: animals were injected with diclofenac 1hr before MES test, divided into 3 subgroups: D1, D2, and D3, with each consisting of 6 animals injected with diclofenac (5, 10, and 20mg/kg).

Diclofenac (10mg/kg) was the medium dose, so it was selected for interaction.

d- combined group: 6 animals were injected with diclofenac 10mg/kg/i.p. 45 minutes later, they were injected with retigabine at 8mg/kg, i.p.15 minutes before the MES test.

2.2.4. Aminopyridine (Kv channel blocker)

Retigabine group

4-aminopyridine (1, 2, and 4mg/kg/s.c.) was injected 10 minutes before retigabine (8mg/kg/i.p.), followed by the MES test after 15 minutes as follows:

- R1, R2, and R3, with each consisting of 6 animals injected with 4-aminopyridine (1, 2, and 4mg/kg).

Diclofenac group

diclofenac (10 mg/kg /i.p.) was injected 50 minutes before 4-aminopyridine (0.5, 1, and 2mg/kg/s.c.).10 minutes later, they were exposed to the MES test as follows:

- D1, D2, and D3, with each consisting of 6 animals injected with 4-aminopyridine (0.5, 1, and 2mg/kg).

Combined group

6 mice injected with diclofenac (10mg/kg/i.p.) 35 minutes before 4-aminopyridine (4mg/kg/s.c.). 10 minutes later, they were injected with retigabine (8mg/kg /i.p.) exposed to the MES test after 15 minutes.

2.3. Statistical analysis

The obtained continuous variables were tabulated as means $\pm$ SE. Comparisons between different groups were made using one way analysis of variance (one-way ANOVA) followed by post-hoc (least significant difference "LSD") tests as described by Armitage and Berry (1994). The categorical variables were expressed as a number percentage (ordinal variables were compared using Chi-square test for trend). The differences were considered to be significant when $p < 0.05$. Statistical Package of Social Sciences (SPSS) computer software (version 16) was used to carry out the statistical analysis.
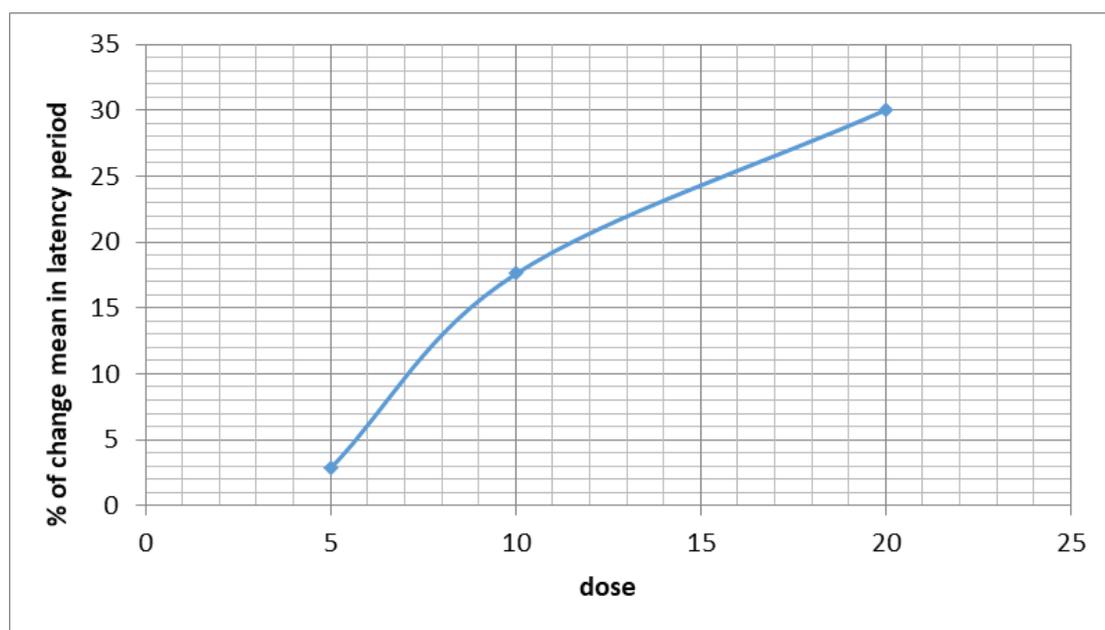
Figure 2: Effect of diclofenac (5, 10, and 20) on percentage of change in mean latency period in picrotoxin-(5mg/kg/i.p.) induced convulsion in mice (model 1).

3. Results

3.1. Effect of diclofenac, retigabine, and their combination on picrotoxin-induced convulsion in mice (model 1)

In the control group, injected with picrotoxin (5mg/kg/i.p.), the mean latency period was 7.92 ± 2.12 minutes. Pre-administration of diclofenac in a dose of 5, 10, and 20mg/kg/i.p. increased the mean latency period to 8.15 ± 0.84 min., 9.31 ± 0.53 min., and 10.3 ± 1.5 min., respectively. Diclofenac (10mg/kg/i.p.) was the medium dose selected for interaction (Figure 2).

Pre-administration of retigabine in a dose of 2mg/kg/i.p. and 4mg/kg/i.p. increased the mean latency period to 35.40 ± 4.6 min. and 37.46 ± 5.63min., respectively. Retigabine in a dose of 8mg/kg/i.p. and 12mg/kg/i.p. completely protected the mice from convulsion (after 180 minutes of observation; after picrotoxin administration no convulsion occurs). Retigabine (4mg/kg/i.p.) was the medium dose selected for interaction (Figure 3).

Effect of combined diclofenac (10mg/kg/i.p.) and retigabine (4mg/kg/i.p.) in picrotoxin- (5mg/kg/i.p.) induced convulsion in mice (model 1)

Combined treatment with both retigabine (4mg/kg/i.p.) and diclofenac (10mg/kg/i.p.) significantly increased the frequency of protection and also significantly decreased the death rate, protecting all mice from convulsion (0% frequency of convulsion) with complete inhibition of death (Table 1).
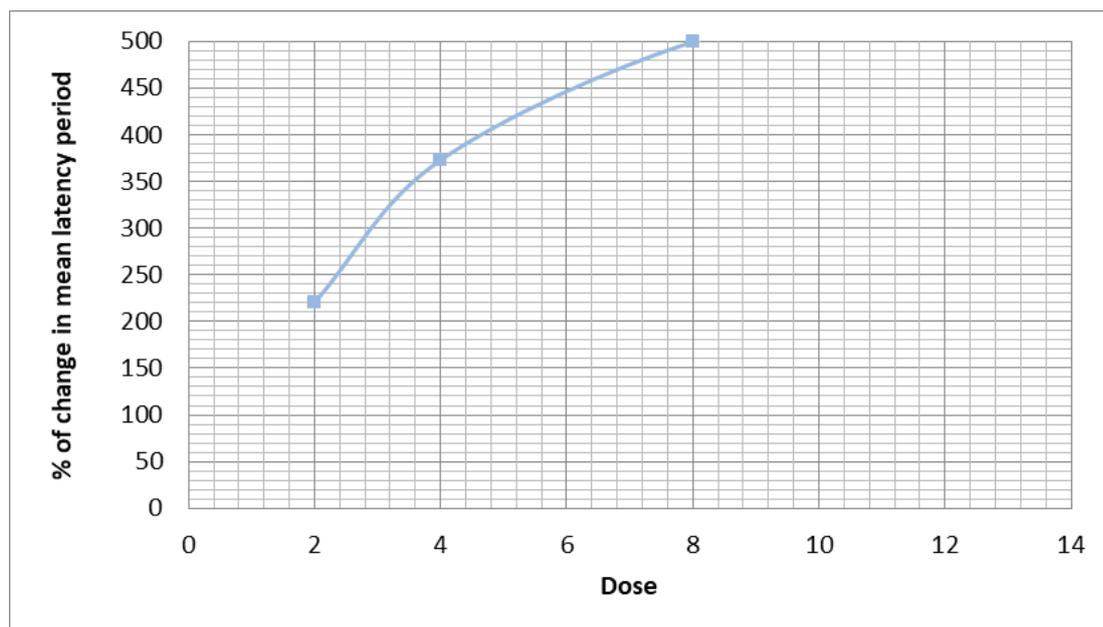
Figure 3: Effect of retigabine (2, 4, 8, and 12mg/kg/i.p.) on percentage of change in mean latency period in relation to control group in picrotoxin- (5mg/kg/i.p.) induced convulsion in mice (model 1).

Table 1: Effect of diclofenac (10mg/kg/i.p.), retigabine (4mg/kg/i.p.), and their combination on mean latency period, frequency of convulsion, frequency of protection, and death rate in picrotoxin- (5mg/kg/i.p.) induced convulsion in mice (model 1).

Group Parameter	Control group picrotoxin (N = 6)	Diclofenac group (N = 6)	Retigabine group (N = 6)	Combined group (N = 6)	P
Latency period (min) Mean $\pm$ SE	7.92 $\pm$ 0.87 [a]	9.31 $\pm$ 0.53 [a]	37.46 $\pm$ 5.63 [b]	No convulsion	$ <0.001**
% of change	—-	17.6%	373%	α	
Frequency of convulsion (%)	100% [a]	100%[a]	50%[b]	0% [c]	# <0.001**
Frequency of Protection (%)	0% [a]	0%[a]	50% [b]	100%[c]	
% of change	—	0	50%	100%	
Death rate N (%)	100% [a]	83.3%[a]	0% [b]	0%[b]	# <0.001**
% of change	——	16.7%	100%	100%	

Groups with the same letters are statistically insignificant (p > 0.05).

[**]highly significant (p < 0.01). $: ANOVA (F) test. #: Chi-square test.

N = the number of animals in each group.

- Mean latency period is the mean period (min) between injection of picrotoxin and onset of convulsion.
- Frequency of convulsion is the number of convulsed animals on the total number of animals.
- Frequency of protection is the number of non-convulsed animals on the total number of animals.

3.2. Effect of diclofenac, retigabine, and their combination in pilocarpine-induced SE in mice (model 2)

The mean latency period in the control group that received pilocarpine (350mg/kg/i.p.) was 9.4 $\pm$ 0.96 minutes. Pre-administration of diclofenac in a dose of 5mg/kg/i.p., 10mg/kg/i.p.,
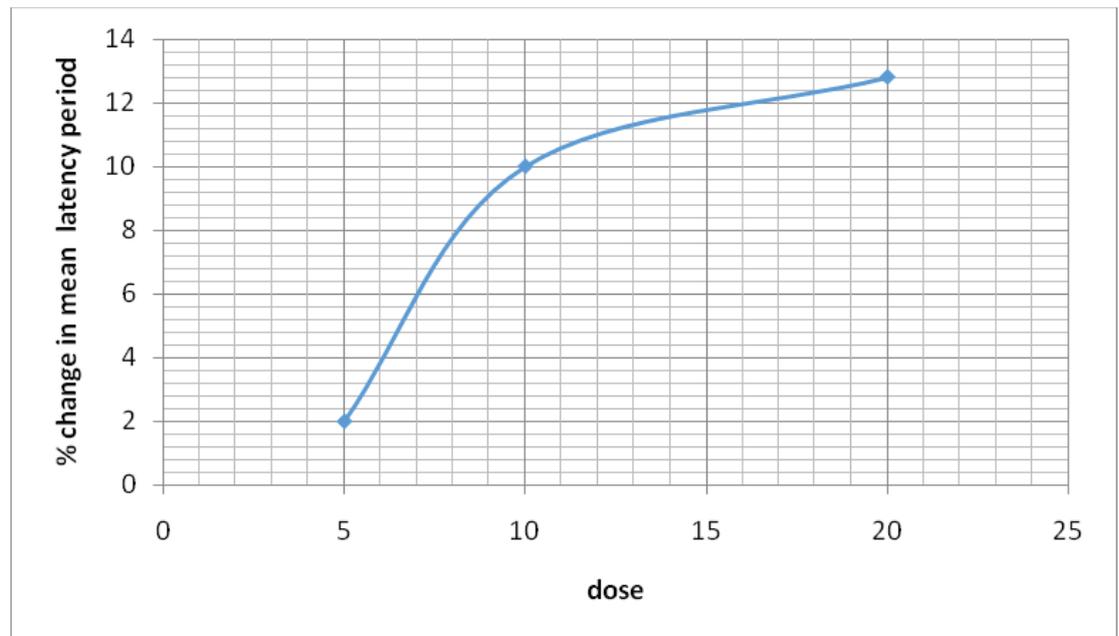
Figure 4: Effect of diclofenac (5, 10, and 20mg/kg/i.p.) on percentage of change in mean latency period in relation to the control group in pilocarpine- (350mg/kg/i.p.) induced sustained epilepsy in mice (model 2).

and 20mg/kg/i.p. increased the mean latency period to 9.60 ± 0.89 min, 10.37 ± 0.92 min, and 10.60 ± 1.6 min., respectively. Diclofenac (10mg/kg/i.p.) was the medium dose, so it was selected for interaction (Figure 4).

Pre-administration of retigabine in a dose of 2mg/kg/i.p., 4mg/kg/i.p., and 8mg/kg/i.p. increased the mean latency period to 13.11 ± 0.12 min., 13.82 ± 0.14 min., and 30.50 ± 4.58 min., respectively, in relation to the control group. Administration of retigabine in a dose of 12mg/kg/i.p. protected all mice from convulsion (after 180 minutes of observation; after pilocarpine administration, no convulsion occurs). Retigabine (8mg/kg/i.p.) was the medium dose, so it was selected for interaction (Figure 5).

Effect of combined diclofenac (10mg/kg/i.p.) and retigabine (8mg/kg/i.p.) in pilocarpine- (350mg/kg/i.p.) induced SE in mice (model 2)

Combined administration of both retigabine 8mg/kg/i.p. and diclofenac 10mg/kg/i.p. significantly increased the mean latency period in relation to the control group and also significantly reduced the frequency of convulsion in relation to all groups to 50%, with non-significant change in death rate (Table 2).

3.3. Effect of diclofenac, retigabine, and their combination in MEST in mice (model 3)

Fixed current of 25MA 0.2 sec. given to the control group of mice produced convulsion in 100 % of the mice. Pre-administration of diclofenac in a dose of 5mg/kg/i.p. before the fixed current protected 16.7% of the mice from convulsion. Diclofenac in a dose of 10mg/kg/i.p.
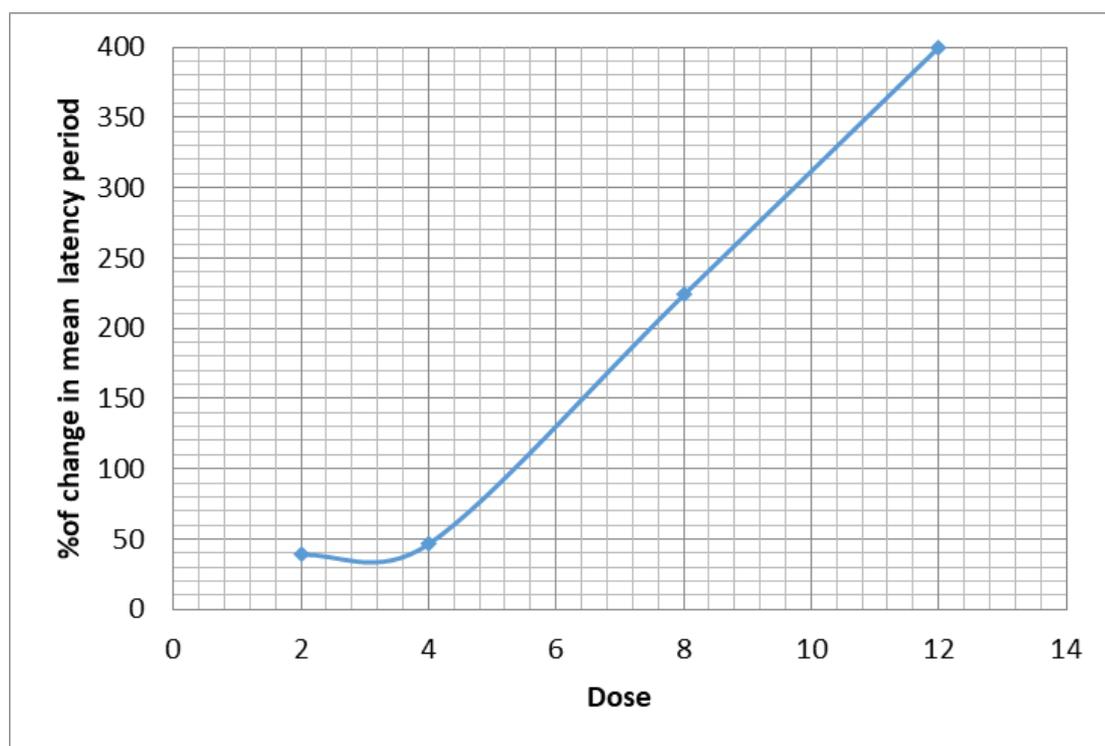
Figure 5: Effect of retigabine (2, 4, 8, and 12mg/kg/i.p.) on the percentage of change in the mean latency period in relation to the control group in pilocarpine- (350mg/kg/i.p.) induced sustained epilepsy in mice (model 2).

Table 2: Effect of diclofenac (10mg/kg/i.p.), retigabine (8mg/kg/i.p.), and their combination on mean latency period, frequency of convulsion, frequency of protection, and death rate in pilocarpine- (350mg/kg/i.p.) induced SE in mice (model 2).

Group Parameter	Control group pilocarpine (N = 6)	Diclofenac group (N = 6)	Retigabine group (N = 6)	Combined group (N = 6)	P
Latency period (min) Mean $\pm$ SE	9.4 $\pm$ 0.96 [a]	10.37 $\pm$ 0.92 [a]	30.50 $\pm$ 4.58 [b]	41 $\pm$ 6.68 [c]	\$ <0.001**
% of change	——	10%	224%	336%	
Frequency of convulsion (%)	100% [a]	100% [a]	66.7% [a]	50% [b]	# 0.04*
Frequency of protection (%)	0% [a]	0% [a]	33.3% [a]	50% [b]	
% of change	——	0	33.3%	50%	
Death rate N (%)	83.3% [a]	83.3% [a]	66.7% [a]	66.7% [a]	# 0.83 NS
% of change	——	0	20%	20%	

Groups with the same letters are statistically insignificant (p > 0.05).

[*]significant (p < 0.05). \$: ANOVA (F) test. #: Chi-square test. NS: non-significant.

N = the number of animals in each group.

• Mean latency period is the mean period (min) between injection of pilocarpine and onset of convulsion.

• Frequency of convulsion is the number of convulsed animals on the total number of animals.

• Frequency of protection is the number of non-convulsed animals on the total number of animals.

and 20mg/kg/i.p. increased percentage of protection from convulsion to 50%. Diclofenac (10mg/kg/i.p.) was the medium dose, so it was selected for interaction (Figure 6).
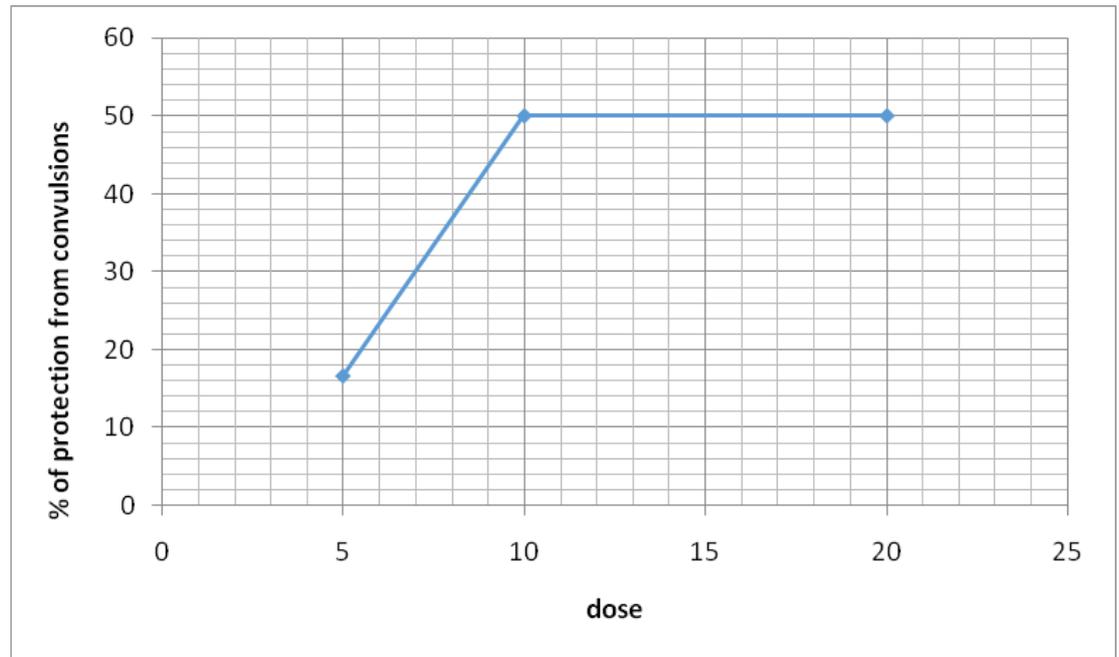
Figure 6: Effect of diclofenac (5, 10, and 20 mg/kg/i.p.) on percentage of protection from convulsion in the maximal electroshock seizure test in mice (model 3).

Pre-administration of retigabine in a dose of 2mg/kg/i.p. and 4mg/kg/i.p. protected 33.3% of the mice from convulsion. Retigabine in a dose of 8mg/kg/i.p. (before the fixed current) increased percentage of protection from convulsion to 50%. Retigabine in a dose of 12mg/kg/i.p. increased percentage of protection from convulsion to 83.3%. Retigabine (8mg/kg/i.p.) was the medium dose, so it was selected for interaction (Figure 7).
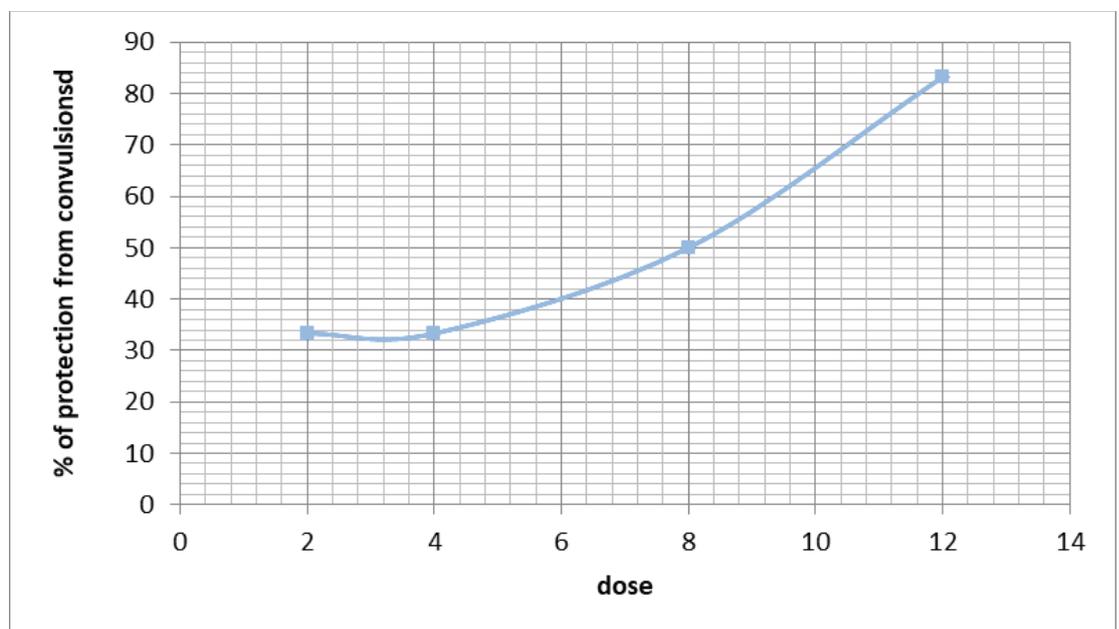

Figure 7: Effect of retigabine (2, 4, 8, and 12 mg/kg/i.p.) on percentage of protection from convulsion in the maximal electroshock seizure test in mice (model 3).

Table 3: Effect of diclofenac (10mg/kg/i.p.), retigabine (8mg/kg/i.p.), and their combination on hind limb extension duration, frequency of convulsion, frequency of protection, and death rate in the MES test in mice model 3.

Group Parameter	Control group (25MA 0. 2S) (N = 6)	Diclofenac group (N = 6)	Retigabine group (N = 6)	Combined group (N = 6)	P
HLE (sec) Mean $\pm$ SE	16.67 ± 1.78 [a]	11.75 ± 1.53 [b]	7 ± 0.75 [b]	no convulsion	\$ <0.001**
% of change	—	29.5%	58%	α	
Frequency of convulsion (%)	100% [a]	50% [b]	50% [b]	0% [c]	# <0.001**
Frequency of protection (%)	0% [a]	50% [b]	50% [b]	100% [c]	
% of change	—	50%	50%	100%	
Death rate (%)	33.3% [a]	33.3% [a]	16.7% [b]	0% [b]	# 0.43 NS
% of change	—	0	50%	100%	

Groups with the same letters are statistically insignificant (p > 0.05).

[*] significant (p < 0.05). \$: ANOVA (F) test. #: Chi-square test. NS: non-significant.

N = the number of animals in each group.

● Mean latency period is the mean period (min) between the injection of picrotoxin and the onset of convulsion.

● HLE hind limb extension duration.

Effect of combined diclofenac (10mg/kg/i.p.) and retigabine (8mg/kg/i.p.) in the MES test in mice (model 3)

Combined administration of both retigabine (8mg/kg/i.p.) and diclofenac (10mg/kg/i.p.) produced complete protection of the mice from convulsions (Table 3).

3.4. Effect of the Kv channel blocker (4-aminopyridine) in the MES test in mice

Effect of 4-aminopyridine (0.5, 1, and 2 mg/kg/s.c) with diclofenac (10 mg/kg/i.p.) in the MES test in mice

Pre-administration of diclofenac in a dose of 10 mg/kg/i.p.in the maximal electroshock model of epilepsy (before the fixed current 25MA 0.2sec.) protected 50% of mice from convulsion. Combined administration of 4-aminopyridine in a dose of 0.5mg/kg/s.c, with a fixed dose of diclofenac 10 mg/kg/i.p., increased the percentage of convulsion to 66.7%. 4-Aminopyridine in a dose of 1mg/kg/s.c, with diclofenac, increased the percentage of convulsion to 83.3%. All tested mice developed convulsion with administration of 4-aminopyridine in a dose of 2mg/kg/s.c with diclofenac. The Kv channel blocker (4-aminopyridine) blocks the efficacy of diclofenac in a dose dependent manner in the maximal electroshock model of epilepsy in mice (Table 4).

Table 4: Effect of 4-aminopyridine (0.5, 1, 2, and 4 mg/kg/s.c) (Kv channel blocker) with diclofenac (10 mg/kg/i.p.) in the maximal electroshock seizure test in mice.

Diclofenac (10mg/kg ,i.p)+4-aminopyridine(0.5,1,2 mg/kg/s.c)	Percentage of convulsion in (MES) model
0.5 mg/kg/s.c	66.7%
1 mg/kg/s.c	83.3%
2 mg/kg/s.c	100%

Table 5: Effect of 4-aminopyridine (1, 2, and 4 mg/kg/s.c) (Kv channel blocker) with retigabine (8 mg/kg/i.p.) in maximal electroshock seizure test in mice.

Retigabine (8 mg/kg /i.p.) +4-aminopyridine (1,2,4 mg/kg/s.c)	Percentage of convulsion in the (MES) model
1 mg/kg/s.c	66.7%
2 mg/kg/s.c	83.3%
4 mg/kg/s.c	100%

Effect of 4-aminopyridine (1, 2, and 4 mg/kg/s.c) with retigabine (8 mg/kg/i.p.) in the maximal electroshock seizure test in mice

Pre-administration of retigabine in a dose of 8 mg/kg/i.p. in the maximal electroshock model of epilepsy (before the fixed current 25MA 0.2s) protected 50% of mice from convulsion. Combined administration of 4-aminopyridine in a dose of 1mg/kg/s.c, with a fixed dose of retigabine 8 mg/kg/i.p., increased the percentage of convulsion to 66.7%. 4-Aminopyridine in a dose of 2mg/kg/s.c, with retigabine, increased the percentage of convulsion to 83.3%. All tested mice (100%) developed convulsion with administration of 4-aminopyridine in a dose of 4mg/kg/s.c with retigabine. The Kv channel blocker (4-aminopyridine) blocks the efficacy of anticonvulsant drug retigabine in a dose dependent manner in the maximal electroshock model of epilepsy in mice (Table 5).

4. Discussion

Epilepsy is the most common serious neurologic disorder that affects 50 million people world-wide. About 25–30% of epileptic patients do not respond well to the generally available classical antiepileptic drugs (AEDs) [8].

AEDs exert their actions either by target voltage-dependent sodium or calcium channels or both, and by their blockade, inhibiting sustained repetitive firing of neurons. Other AEDs are GABA enhancers or reduce glutamate-induced excitation at the receptor level [23].

The intensive pharmacological search for newer AEDs along with clinical trials led to the development of a number of drugs that are primarily used in the form of an add-on therapy of patients with refractory epilepsy [2].

Although the existing AEDs affect various targets in the central nervous system, there are still problems in about 30% of epileptic patients to sufficiently control their seizure activity.

Consequently, the novel targets are searched for the better management of refractory epilepsy [30].

In the present study, we aimed at studying the spectrum of the new anticonvulsant drug retigabine, and its combination with diclofenac, and at investigating the possible mechanisms of action using the three models of convulsion picrotoxin-induced seizures, pilocarpine-induced SE models, and maximal electro shock seizure test (MES).

Retigabine represents a novel antiepileptic drug possessing a completely different mechanism of action when compared to the existing classical and newer antiepileptic drugs. Retigabine acts primarily through opening neuronal potassium channels (KCNQ2 [Kv7.2] and KCNQ3 [Kv7.3]). This stabilizes the resting membrane potential and controls the sub-threshold electrical excitability in neurons, thus preventing the initiation of epileptiform action potential bursts [12]. The KCNQ2 and KCNQ3 channel proteins underlie the neuronal "M-current," inactivating potassium current. "M-currents" are able to act as a brake for repetitive action potential firing and reduce neuronal responsiveness to synaptic input [10].

Picrotoxin acts as non-competitive channel blocker for $GABA_A$ receptors chloride channel. As GABA is an inhibitory neurotransmitter, infusion of picrotoxin inhibits GABA binding to its catalytic receptor; thereby, it has stimulant and convulsant effect [22].

The present study showed that, in the picrotoxin model, retigabine significantly increased the mean latency period in relation to the control group; also retigabine significantly increased the frequency of protection from convulsion, with significant reduction in the death rate. Retigabine in a dose of 8mg/kg/i.p. significantly increased the percentage of protection from convulsion producing 100% protection.

These results are in line with those by Apostolova et al. (2014) who showed that retigabine when administered orally in rat inhibited generalized seizure in acute PTZ model. Electroconvulsive seizures are particularly sensitive to drugs blocking sodium channels. The MES test predicts drugs useful for generalized tonic–clonic seizures and, to a certain extent, for partial secondarily generalized convulsion [14].

The present study showed that, in the MES model, retigabine significantly increased the frequency of protection of the mice from tonic hind limb extension with significant reduction in both HLE duration and death rate.

These results also matched those of Apostolova et al. (2014) who showed that retigabine when administered orally in rat inhibited generalized seizure in MES test at 18mg/kg/p.o. (30 min pretreatment time).

Pilocarpine-induced status epilepticus model is initiated via muscarinic receptors and further mediated via NMDA receptors. The sustained increase in extracellular glutamate levels after pilocarpine infusion is related to limbic seizures. Hippocampal dopamine release induced by pilocarpine may be functionally important in epileptogenesis [28].

In pilocarpine-induced sustained epilepsy model, pre-administration of retigabine in a dose of 8mg/kg/i.p. significantly increased the mean latency period percentage of change in relation to the control group. Regarding the frequency of protection and death rate, no significant change was found between retigabine and control group. Retigabine 12mg/kg/i.p. significantly increased the percentage of protection from convulsion.

These results are in agreement with those of Large et al. (2012) who reported that the effects of retigabine in a range of animal models of seizures induced by intracerebroventricular NMDA were inhibited with retigabine, an ED50 of 9.1 mg/kg, i.p (15 min pretreatment time).

According to this study, retigabine is found to be effective in the three models of convulsion, which explain the broad spectrum of its anticonvulsant activity.

In this work, to confirm that the efficacy of retigabine in these models is likely to be mediated by activation of KCNQ (Kv7) channels, anticonvulsant efficacy of retigabine 8mg/kg/i.p. in the MES seizure model was blocked in a dose dependent manner by the voltage gated potassium channels (Kv) blocker 4-aminopyridine (1, 2, and 4mg/kg/s.c).

These results are in agreement with those of Sankar et al. (2009) who demonstrated that XE-991 dihydrochloride, which is a potent and selective blocker of KCNQ voltage-gated potassium channels, that blocks KCNQ2/ KCNQ3 M-currents, reduced the anticonvulsant efficacy of retigabine in the electroshock seizure model in mice. As regards this study, retigabine was more effective in the picrotoxin model in a dose of 4mg/kg/i.p. and MES in dose of 8mg/kg/i.p. than in the pilocarpine model (not effective in dose of 8mg/kg/i.p. while producing effect with 12mg/kg/i.p.). Retigabine was most effective in the picrotoxin model. This could be explained by its action on voltage gated k ion channel in addition to its effect on GABA neurotransmission in the $GABA_A$ receptors by increasing the chloride conductance.

These results are consistent with the finding of Treven et al. (2015) who concluded that, apart from increasing the concentration of GABA in the brain by either enhancing GABA synthesis or blocking GABA metabolism, retigabine allosterically potentiates GABA induced current in rats' cortical neurons in a concentration dependent manner. In addition, Rundfeldt and Netzer (2000) showed that a retigabine in vitro study augmented the synthesis of GABA in rat hippocampal brain slices.

NSAIDs are widely used for anti-inflammatory, analgesic and antipyretic activities. They produce their effects through the inhibition of the cyclooxygenase enzyme (COX), which is responsible for converting the arachidonic acid into prostaglandins (PGs) and proinflammatory mediators. The COX enzyme exists in two isoforms; namely, COX-1 is expressed constitutively in most tissues, while COX-2 is inducible during inflammatory response [6].

In the MES model, diclofenac showed a significant decrease in hind limb extension duration in relation to the control group; also diclofenac produced significant protection from convulsion accompanied with non-significant change in death rate.

These results are in accordance with those of Bhuvaneshwari (2015) who demonstrated that administration of diclofenac (10 mg/kg/i.p.) one hour before the MES model in mice potentiated the effect of nimodipine when administrated together causing significant reduction in hind limb extension duration with rapid recovery compared with that of nimodipine alone.

These results also are in the same line to what was reported by Peretz et al. (2005) who showed that intraperitoneal injection of diclofenac at different doses either 30 min or 2 hours before electroshock showed significant protection from convulsion in a dose dependent manner.

The results of the present work showed that administration of diclofenac (10mg/kg/i.p.) in the picrotoxin model produced non-significant increase in the mean latency period in relation to the control group. In addition, all tested mice developed convulsion with 100% percentage of convulsion and non-significant decrease in death rate.

These results could be in line with those of Almaghour et al. (2014) who concluded that diclofenac administrated 30 min before picrotoxin injection had no effect on episodes of convulsions, while celecoxib (selective COX-2 inhibitor) significantly reduced the episodes. This could be explained by weak inhibition of diclofenac to PGE2, which enhanced the release of glutamate from the nerve terminals and astrocytes, which is an excitatory neurotransmitter, also leading to a decrease in the brain GABA levels resulting in convulsion.

On the contrary, Mirhadi (2012) concluded that NSAIDs, like diclofenac, were found to increase the latency to onset of PTZ-induced seizures in mice that appeared through a potentiating effect of these NSAIDs on concomitantly administered antiepileptic drugs attributing this effect to their potential blockade of PGs synthesis.

The present work showed that, in pilocarpine-induced sustained epilepsy in mice, administration of diclofenac produced non-significant increase in the mean latency period. All tested mice exhibited tonic-clonic convulsion with non-significant change in death rate.

These results are in line with Radua et al. (2017) who reported that, in the pilocarpine model, diclofenac is neuroprotective, reduces mossy fiber sprouting, and diminishes P-glycoprotein upregulation, which is responsible for resistance to antiepileptic drugs, but rarely protects against seizures.

Diclofenac 10 mg/kg/i.p. was effective in MES, while it was not effective in the picrotoxin and pilocarpine models, which could be explained by its ability to act as a potassium channel opener. This explanation could be supported by Peretz et al. (2005) who reported that diclofenac acts as (KNCQ2/ KNCQ3) opener and depresses cortical neuron activity.

As a result of the chronic nature of epilepsy, patients often require an additional therapy for other underlying diseases, and thus, drug interactions may occur. Unexpected effect and alterations in pharmacological actions are common risks of drug interactions that may lead, sometimes, to hospital admission [16]. NSAIDs, as diclofenac, are prescribed to, and may be taken by, patients who suffer from epilepsy in several cases as headache and low back pain.

The current work revealed that combined administration of retigabine and diclofenac caused significant protection against convulsions induced by the picrotoxin model and against tonic hind limb extension induced by the MES test, as compared to the control group and to each of the retigabine and the diclofenac groups. This combination completely protected mice from convulsion. Furthermore, that combination significantly prolonged the mean latency period of convulsion in pilocarpine-induced sustained epilepsy model as compared to the control group and to each of the retigabine and the diclofenac groups.

These results are in line with those of Peretz et al. (2005) who reported that an in vitro study in rat cortical neurons, using both diclofenac and meclofenamic acid (fenamates), caused shifting the voltage dependence of KCNQ2/3 channel activation curve to more hyperpolarized potentials and slow channel deactivation. As a result of this leftward shift of the KCNQ2/Q3 activation curve, diclofenac leads to a progressive hyperpolarization of the resting membrane potential, enhancing neuronal M-currents stabilizing the KCNQ2/Q3 channel in the open state. In conclusion, diclofenac is a newer opener of the heterologously expressed KCNQ2/3 channels, which can explain the potentiation of diclofenac to retigabine (Kv channel opener) as anticonvulsant being acting on the same subtype of potassium channel.

As regards this study, confirmation for possible action of diclofenac is a template of KCNQ2/3 potassium channel opener and exhibits anticonvulsant properties; the KV channel blocker 4

aminopyridine (0.5, 1, and 2mg/kg/s.c.) blocked the efficacy of diclofenac (10mg/kg/i.p.) in the maximal electroshock test in a dose dependent manner increasing percentage of convulsion.

According to the results of the current study, we concluded that retigabine is a first-in-class antiepileptic drug AED that reduces neuronal excitability by enhancing the activity of KCNQ (Kv7) potassium ion channels and was proved to have a broad spectrum antiepileptic effects and diclofenac (a NSAID) was proved to potentiate retigabine action suggesting a new mechanism of action for diclofenac as an opener of potassium (KCNQ2/3) channels.

Competing Interests

The authors declare no competing interests.

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