International Journal of Medical and Pharmaceutical Research

Online ISSN-2958-3683 | Print ISSN-2958-3675
Frequency: Bi-Monthly
Available online on: https://ijmpr.in/

Original Article

Evaluation of Dose-Dependent Anticonvulsant Effects of Nifedipine in Pilocarpine-Treated Zebrafish

Aakash A[1], Nivedhitha R[2*], Abivarshini R[1], Venkadesh K[1], Akash A[1], Amitha Jane J[1], Vimalkumar G[1]

[1]CRMI, Dhanalakshmi Srinivasan Medical College & Hospital, Siruvachur, Perambalur, Tamil Nadu.
[2]Assistant Professor, Department of Anaesthesiology, Dhanalakshmi Srinivasan Medical College & Hospital, Siruvachur, Perambalur, Tamil Nadu.

Corresponding Author:

Dr. Nivedhitha. R
Assistant Professor Department of Anaesthesiology Dhanalakshmi Srinivasan Medical College & Hospital, Siruvachur Perambalur-621113 Tamil Nadu
Emil:
nivedhithaakshaya@gmail.com

Received: 01-04-2026
Accepted: 16-05-2026
Available online: 19-05-2026

ABSTRACT

Background: Epilepsy is a chronic neurological disorder with recurrent seizures, often refractory to existing antiepileptic drugs, necessitating exploration of novel therapeutic targets. Calcium channel modulation is increasingly recognised in seizure control. Nifedipine, an L-type calcium channel blocker, has shown potential anticonvulsant effects in experimental models, but its dose-dependent efficacy in pilocarpine-induced seizures remains insufficiently studied.
Materials and Methods: This experimental study evaluated the dose-dependent anticonvulsant effects of nifedipine in a pilocarpine-induced seizure model in adult zebrafish (Danio rerio). Fish were acclimatised and randomly assigned to control, pilocarpine, diazepam standard, and nifedipine-treated groups (5, 10, and 20 mg/L). Seizures were induced using pilocarpine, and behavioural parameters, including seizure latency, seizure severity score (0–5 scale), seizure duration, and mortality, were recorded. Statistical analysis was performed using one-way ANOVA and appropriate post hoc tests.
Results: Pilocarpine induced severe seizure activity characterised by reduced latency, maximal seizure scores, prolonged seizure duration, and 35% mortality. Diazepam significantly suppressed seizures and prevented mortality. Nifedipine produced a significant dose-dependent anticonvulsant effect, increasing seizure latency (65.6–140 s), reducing seizure severity (3.65–0.55), shortening seizure duration (130.6–38.65 s), and decreasing mortality (20%–5%). The highest dose showed effects comparable to diazepam.
Conclusion: Nifedipine demonstrated significant dose-dependent anticonvulsant and neuroprotective effects in a zebrafish seizure model. These findings suggest that calcium channel blockade may represent a promising adjunct or alternative therapeutic strategy for epilepsy, warranting further preclinical and translational studies.

Keywords: *Epilepsy, Nifedipine, Zebrafish, Pilocarpine, Anticonvulsant, Calcium channel blocker, Seizure model.*

INTRODUCTION

Epilepsy is a long-term neurological disorder characterised by recurrent seizures caused by abnormal electrical activity in the brain. Due to its effects on physical health, emotional well-being, and general quality of life, it continues to be a significant health concern for millions of people worldwide. There is a need for safer and more effective treatment options because many patients still do not achieve complete seizure control despite the availability of various antiepileptic medications. [1,2] Calcium ions play a crucial role in controlling nerve impulse transmission and neuronal function. Neuronal hyperexcitability and the onset of seizures may be influenced by increased calcium entry via voltage-gated calcium channels. Through blockade of L-type calcium channels, nifedipine, a dihydropyridine calcium channel blocker commonly used in cardiovascular disorders such as hypertension and angina, has been shown in experimental studies to possess

anticonvulsant and neuroprotective properties. [3,4]. Zebrafish (Danio rerio) are widely used as an experimental model in neurological research due to their genetic similarity to humans, ease of maintenance, rapid reproduction, and suitability for drug screening studies. Pilocarpine-induced seizure models in zebrafish are frequently used to evaluate potential anticonvulsant agents, as they share features with human temporal lobe epilepsy. [5–8] However, few studies have evaluated the dose-dependent anticonvulsant effects of nifedipine in a pilocarpine-induced zebrafish seizure model. Therefore, the present study was designed to assess the anticonvulsant effects of different doses of nifedipine in pilocarpine-treated zebrafish using behavioural and locomotor parameters.

Aim of the study

The present study aimed to evaluate the dose-dependent anticonvulsant effects of nifedipine in a pilocarpine-induced seizure model in zebrafish.

Objectives

Primary objective

- To evaluate the anticonvulsant activity of nifedipine in a pilocarpine-induced seizure model in zebrafish.

Secondary objectives

- To assess the effect of nifedipine on seizure latency.
- To assess the effect of nifedipine on seizure severity score.
- To assess the effect of nifedipine on seizure duration.
- To evaluate mortality associated with pilocarpine-induced seizures following nifedipine treatment.
- To determine the dose-dependent anticonvulsant response of nifedipine.

MATERIALS AND METHODS

Study design

This experimental study was undertaken to investigate the dose-dependent anticonvulsant activity of Nifedipine against Pilocarpine-induced seizures in zebrafish.

Study setting

The study was conducted in Research Lab, Department of Pharmacology, Dhanalakshmi Srinivasan Medical College and Hospital, Siruvachur under controlled laboratory conditions.

Experimental animals

Healthy adult zebrafish (*Danio rerio*) of either sex were selected for the study. The fish measured approximately 3–4 cm in length and weighed around 0.3–0.5 g. They were obtained from an authorised aquarium supplier and allowed to acclimatise to laboratory conditions for two weeks before the start of the experiment.

The zebrafish were maintained in clean glass tanks containing dechlorinated water under standard environmental conditions. The water temperature was maintained at 26 ± 2°C with a pH range of 6.8–7.5. A 14-hour light and 10-hour dark cycle was followed throughout the study period. The fish were fed twice daily with commercially available fish feed, and feeding was withheld overnight before experimentation. [9]

Ethical approval

The study protocol was reviewed and approved by the Institutional Animal Ethics Committee (IAEC) with reference 022/DSMCH/IAEC/RP/2025. All experimental procedures were carried out in accordance with standard ethical guidelines for the care and handling of laboratory animals.

Grouping of animals

The zebrafish were randomly divided into six groups, each containing an equal number of fish.

- Group I: Normal saline
- Group II: Pilocarpine 300mg/L
- Group III: Diazepam 5mg/L
- Group IV: Nifedipine 5mg/L
- Group V: Nifedipine 10mg/L
- Group VI: Nifedipine 20mg/L

Experimental procedure

Before the experiment, nifedipine was prepared in graded concentrations and used for pre-treatment. Zebrafish in the treatment groups were exposed (immersed) to the respective concentrations of nifedipine for a predefined pre-incubation period before pilocarpine administration to evaluate its protective anticonvulsant effects. The standard group consisted of fish treated with diazepam. Pilocarpine solution was used to induce seizure-like activity in zebrafish as per established

experimental protocols and validated epilepsy models.[10,11] This model replicates status epilepticus through muscarinic receptor activation, leading to sustained neuronal hyperexcitability. Each fish was monitored separately for the emergence of seizure-like behaviour following pilocarpine injection.

Assessment of anticonvulsant activity

Seizure-related behavioural changes were observed and recorded following pilocarpine exposure. The parameters assessed included seizure latency, seizure severity score, seizure duration, and mortality. Seizure latency was defined as the time taken for the appearance of the first seizure-like behaviour after pilocarpine administration. Seizure severity was graded using a behavioural scoring scale ranging from 0 to 5 based on swimming pattern and motor abnormalities. [12] A behavioural grading system based on swimming pattern and motor impairments was used to evaluate the severity of seizures.

- **Score 0:** Normal swimming behaviour
- **Score 1:** Increased swimming activity
- **Score 2:** Jittery movement at the top of the tank
- **Score 3:** Ataxia/Hyperactivity
- **Score 4:** Circular swimming, circling a small area
- **Score 5:** Erratic burst movement with loss of posture/ cockscrew swimming [13,14].

Seizure duration was measured from seizure onset until recovery. Mortality observed during the experimental period was recorded and expressed as percentage mortality.

Statistical analysis

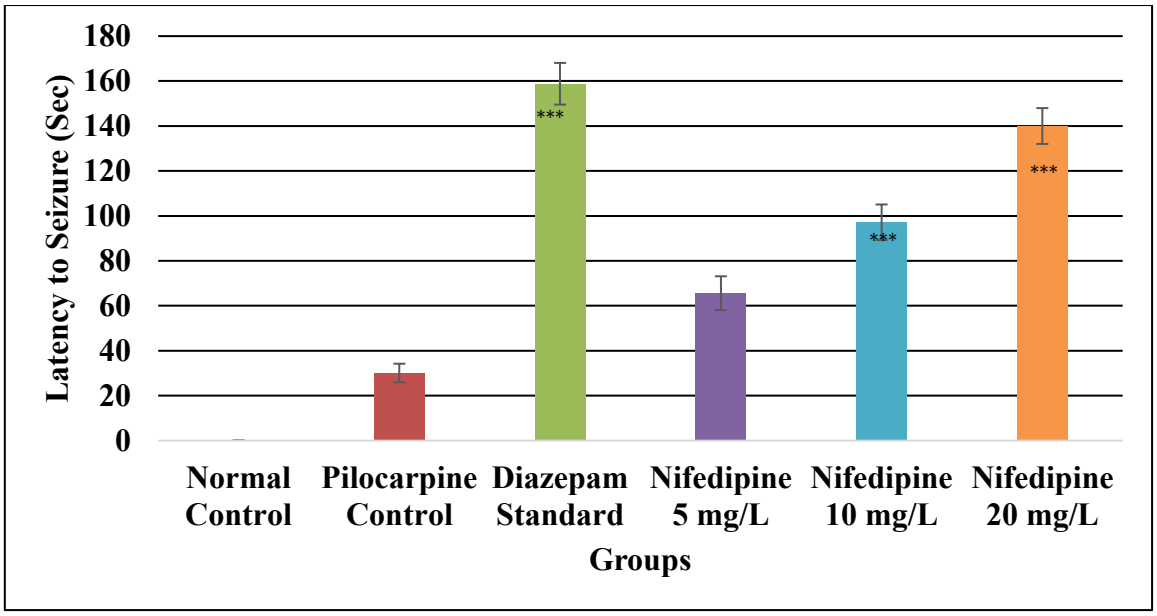
Data were expressed as mean ± sd. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by appropriate post hoc analysis, the Scheffé t-test. A p-value <0.05 was considered statistically significant at 95% confidence interval. SPSS 20.0 version was used for analysis.

RESULTS

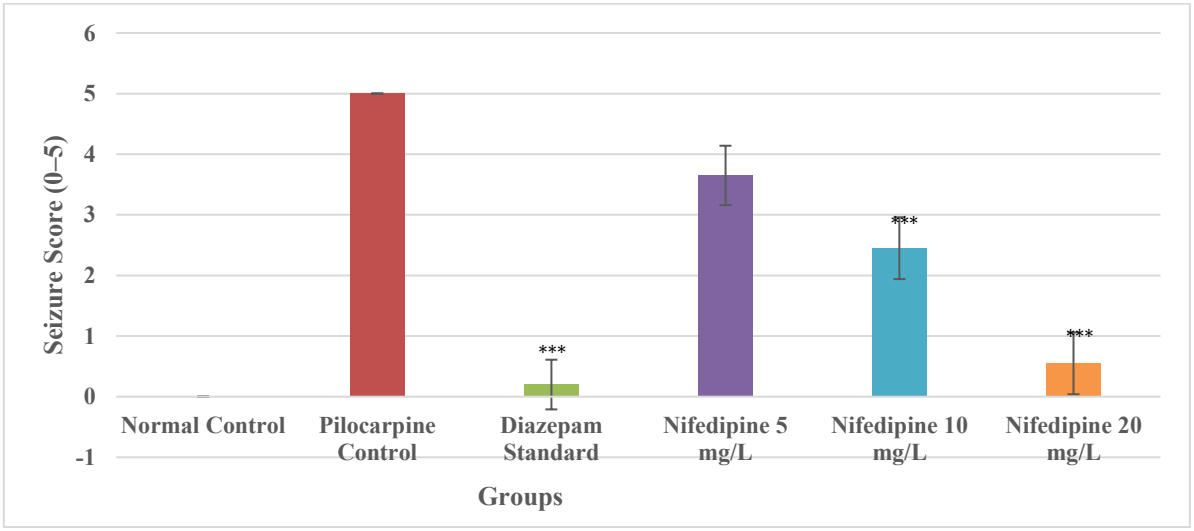
Table 1 summarises the anticonvulsant effect of nifedipine against pilocarpine-induced seizures in zebrafish. Pilocarpine administration produced marked seizure activity, characterised by reduced seizure latency, increased seizure severity, prolonged seizure duration, and elevated mortality. Diazepam significantly attenuated all seizure parameters and completely prevented mortality. Nifedipine demonstrated dose-dependent neuroprotective and anticonvulsant effects, with progressive improvement in all assessed parameters. The highest dose (20 mg/L) exhibited effects comparable to diazepam. One-way ANOVA revealed a highly significant difference in seizure latency among groups ($F(5,114) = 1604.54$, $p < 0.001$). Post hoc Tukey analysis showed significant differences between pilocarpine control and all treatment groups ($p < 0.001$). The pilocarpine group exhibited the shortest latency (30.10 ± 4.14 s), whereas diazepam produced the longest latency (158.80 ± 9.29 s). Nifedipine significantly increased latency in a dose-dependent manner (Graph 1). Seizure severity scores differed significantly between groups (Kruskal–Wallis $H = 110.89$, $p < 0.001$). Both diazepam and higher doses of nifedipine significantly reduced seizure scores compared with pilocarpine control (Graph 2). Seizure duration showed a significant difference across groups ($F = 1569.57$, $p < 0.001$), with nifedipine producing a dose-dependent reduction in seizure duration relative to pilocarpine control (Graph 3). Mortality was highest in the pilocarpine control group (35%), while no mortality was observed in the normal control and diazepam groups. Nifedipine reduced mortality in a dose-dependent manner, with rates of 20%, 10%, and 5% at 5, 10, and 20 mg/L, respectively (Graph 4).

Table-1: Effect of nifedipine on pilocarpine-induced seizures in zebrafish

Groups	Dose/drug	Latency (sec) (MEAN±SD)	Seizure Score (MEAN±SD)	Duration (sec) (MEAN±SD)	Mortality	
					n	%
Group-I	Normal saline	0.00±0.00	0.00± 0	0.00±0.00	0	0.00
Group-II	Pilocarpine (300mg/L)	30.10±4.14	5 ± 0	261.75 ± 18.54	7	35.00
Group-III	Diazepam (5mg/L)	158.80± 9.29	0.2 ± 0.41	17.9 ± 5.08	0	0.00
Group-IV	Nifedipine (5 mg/L)	65.63±7.54	3.65 ± 0.49	130.6 ± 14.83	4	20.00
Group-V	Nifedipine (10 mg/L)	97.15±7.94	2.45 ± 0.51	82.8 ± 7.89	2	10.00
Group-VI	Nifedipine (20 mg/L)	140.03±7.97	0.55 ± 0.51	38.65 ± 8.29	1	5.00



Graph-1: Effect of Nifedipine on Latency to Seizure



Graph-2: Effect of Nifedipine on Seizure Score

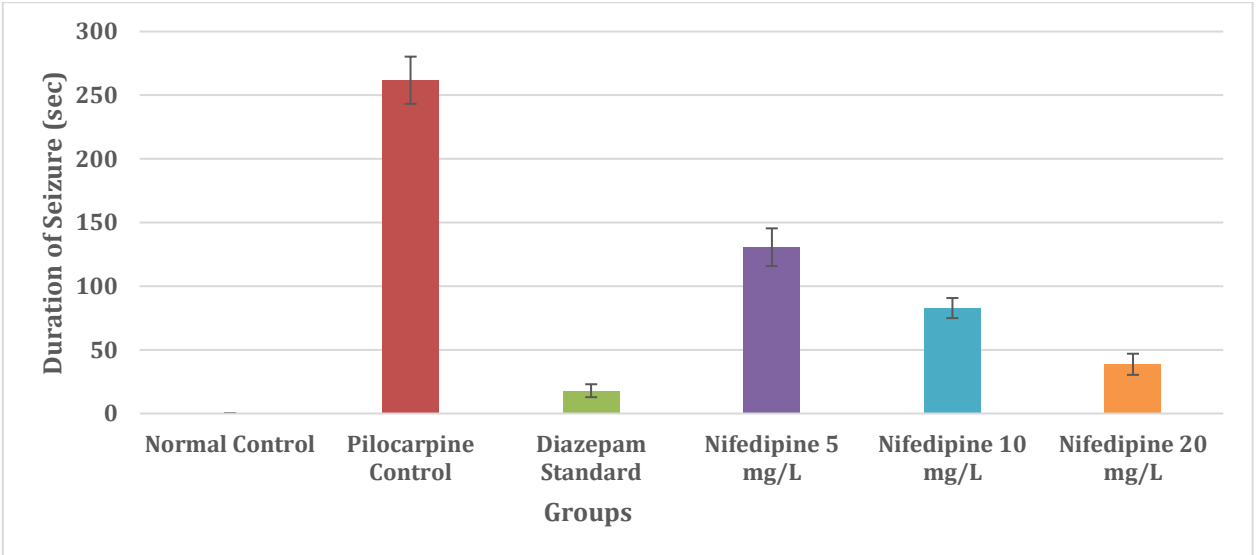
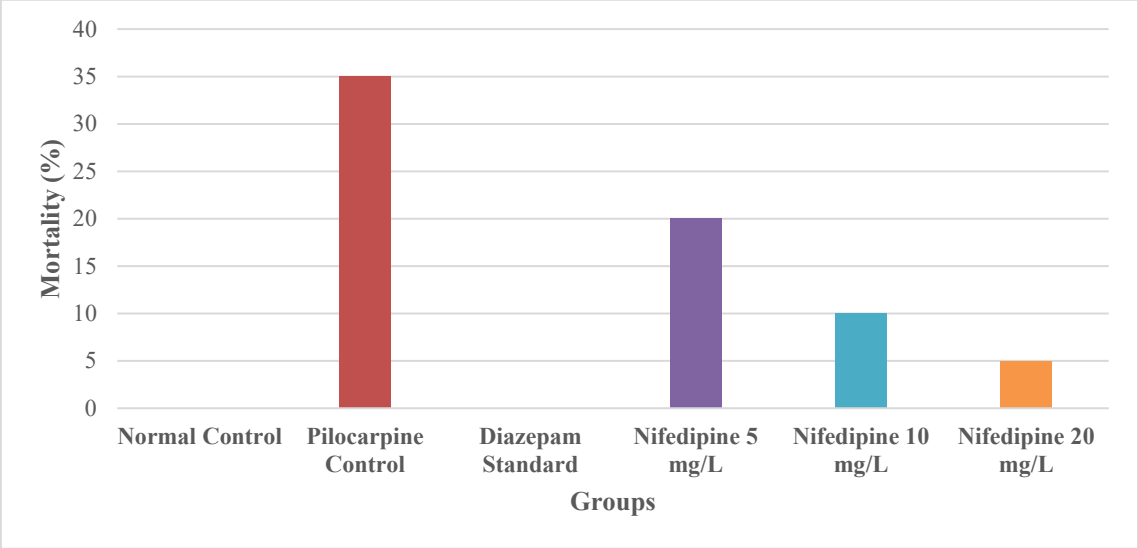


Figure 3: Effect of Nifedipine on Duration of Seizures



Graph 4: Effect of Nifedipine on Mortality

DISCUSSION

The present study demonstrates that nifedipine exhibits a significant dose-dependent anticonvulsant effect in a pilocarpine-induced seizure model in zebrafish. Pilocarpine administration produced marked seizure activity characterised by reduced seizure latency, increased seizure severity, prolonged seizure duration, and elevated mortality, consistent with established experimental models of status epilepticus and temporal lobe epilepsy. The seizure pattern observed in the pilocarpine control group closely matches previous experimental findings where pilocarpine induces status epilepticus through muscarinic receptor activation, leading to sustained cholinergic overexcitation and neuronal hyperexcitability. [10,11] In the present study, this was reflected by a significantly reduced seizure latency (30.10 ± 4.14 s), maximal seizure severity score (5), prolonged seizure duration (261.75 ± 18.54 s), and elevated mortality (35%), confirming successful induction of a robust epileptic model. In contrast, nifedipine produced a clear dose-dependent anticonvulsant response. Seizure latency progressively increased with dose (65.6 ± 7.54 s at 5 mg/L to 140 ± 7.97 s at 20 mg/L), while seizure severity decreased (3.65 ± 0.49 to 0.55 ± 0.51), and seizure duration was significantly reduced (130.6 ± 14.83 s to 38.65 ± 8.29 s). Mortality also decreased in a graded manner from 20% to 5%, indicating a strong neuroprotective effect.

These findings are statistically supported by significant differences across groups, including seizure latency ($F(5,114) = 1604.54$, $p < 0.001$), seizure severity (Kruskal–Wallis $H = 110.89$, $p < 0.001$), and seizure duration ($F = 1569.57$, $p < 0.001$), confirming a consistent pharmacological effect of nifedipine across multiple seizure parameters. The observed anticonvulsant activity of nifedipine is consistent with evidence that calcium channel blockade reduces neuronal excitability and seizure susceptibility.[3,4] Calcium channel blockers have been shown to attenuate seizure severity and reduce neuronal injury in chemically induced epilepsy models, supporting the findings of the present study.[4] The present results extend this evidence by demonstrating a clear graded pharmacological response of nifedipine in a zebrafish seizure model, with higher doses showing effects approaching those of diazepam. The behavioural seizure patterns observed in this study are consistent with established zebrafish epilepsy models, which describe hyperlocomotion, clonus-like activity, and loss of posture following convulsant exposure. [6,7] The behavioural scoring system used in this study aligns with validated zebrafish seizure grading methods, confirming the suitability of this model for anticonvulsant screening.[8]

Diazepam significantly suppressed seizure activity and completely prevented mortality, consistent with its established GABA-A mediated anticonvulsant mechanism. In comparison, nifedipine demonstrated significant but partial seizure suppression, with the highest dose showing effects comparable to diazepam in seizure latency and severity reduction. This suggests that calcium channel blockade may provide an alternative or complementary mechanism for seizure control. Pilocarpine-induced seizures resulted in the highest mortality rate (35%), consistent with severe status epilepticus conditions.[10] Nifedipine reduced mortality in a dose-dependent manner, suggesting a neuroprotective effect against seizure-induced systemic dysfunction. This is in agreement with experimental evidence indicating that calcium channel inhibition may reduce neuronal injury and improve survival outcomes in seizure models. [4] The anticonvulsant effect of nifedipine may be attributed to the blockade of L-type voltage-gated calcium channels, resulting in reduced calcium influx and decreased neuronal excitability. [3] Excess intracellular calcium is a key contributor to excitotoxicity and seizure propagation; thus, its inhibition likely plays a central role in the observed anticonvulsant and neuroprotective effects. [4]

However, the present study has limitations. Molecular biomarkers of oxidative stress, neuronal injury were not assessed. Additionally, pharmacokinetic profiling of nifedipine in zebrafish was not performed. Further studies are required to

elucidate the underlying molecular mechanisms and validate the translational potential of nifedipine in mammalian seizure models.

CONCLUSION

The administration of Nifedipine has shown dose-dependent anticonvulsant activity in the pilocarpine-induced seizure model in zebrafish. This drug is effective at increasing seizure latency, reducing seizure severity and duration, as well as decreasing mortality rate in a dose-dependent manner. The maximal dose produced similar results to diazepam, indicating anticonvulsant and neuroprotective activity. These results indicate that nifedipine can be potentially repurposed as a therapeutic drug for seizures, but more studies need to be conducted.

Conflict of interest: The authors declare no conflict of interest.

Funding: Self

REFERENCE

1. World Health Organization. Epilepsy [Internet]. Geneva: World Health Organization; 2024 [cited 2026 May 7]. Available from: https://www.who.int/health-topics/epilepsy#tab=tab_1
2. Fisher RS, Acevedo C, Arzimanoglou A, Bogacz A, Cross JH, Elger CE, Engel J Jr, Forsgren L, French JA, Glynn M, Hesdorffer DC, Lee BI, Mathern GW, Moshé SL, Perucca E, Scheffer IE, Tomson T, Watanabe M, Wiebe S. ILAE official report: a practical clinical definition of epilepsy. Epilepsia. 2014 Apr;55(4):475-82. doi: 10.1111/epi.12550. Epub 2014 Apr 14. PMID: 24730690.
3. Rogawski MA. Diverse mechanisms of antiepileptic drugs in the development pipeline. Epilepsy Res. 2006 Jun;69(3):273-94. doi: 10.1016/j.eplepsyres.2006.02.004. Epub 2006 Apr 18. PMID: 16621450; PMCID: PMC1562526.
4. Saniya K, Patil BG, Madhavrao C, Prakash KG. Effect of calcium channel blockers on the seizures, oxidative stress, and histoarchitecture in the rats. J Pharmacol Pharmacother 2019;10:93-9.
5. Durairaj, B., & Manish, R. (2019). Zebrafish: An In Vivo Model For The Study Of Therapeutic Targets Of Epilepsy. *NeuroRegulation*, *6*(4), 190. https://doi.org/10.15540/nr.6.4.190
6. Gawel, Kinga & Langlois, Mélanie & Martins, Teresa & Ent, Wietske & Tiraboschi, Ettore & Jacmin, Maxime & Crawford, Alexander & Esguerra, Camila. (2020). Seizing the moment: Zebrafish epilepsy models. Neuroscience & Biobehavioral Reviews. 116. 10.1016/j.neubiorev.2020.06.010.
7. Paudel YN, Kumari Y, Abidin SAZ, Othman I, Shaikh MF. Pilocarpine-Induced Behavioural and Biochemical Alterations in Chronic Seizure-Like Condition in Adult Zebrafish. Int J Mol Sci. 2020 Apr 3;21(7):2492. doi: 10.3390/ijms21072492. PMID: 32260203; PMCID: PMC7178024.
8. Szep D, Dittrich B, Gorbe A, Szentpeteri JL, Aly N, Jin M, Budan F, Sik A. A comparative study to optimize experimental conditions of pentylenetetrazol and pilocarpine-induced epilepsy in zebrafish larvae. PLoS One. 2023 Jul 28;18(7):e0288904. doi: 10.1371/journal.pone.0288904. PMID: 37506089; PMCID: PMC10381053.
9. Sakai C., Ijaz S., Hoffman E.J. Zebrafish Models of Neurodevelopmental Disorders: Past, Present, and Future. Front. Mol. Neurosci. 2018;11:294. doi: 10.3389/fnmol.2018.00294.
10. Vezzani A. Pilocarpine-induced seizures revisited: what does the model mimic? Epilepsy Curr. 2009 Sep-Oct;9(5):146-8. doi: 10.1111/j.1535-7511.2009.01323.x. PMID: 19826509; PMCID: PMC2759049.
11. Smoulders, I., Khan, G.M., Manil, J., Ebinger, G. and Michotte, Y. (1997), NMDA receptor-mediated pilocarpine-induced seizures: characterization in freely moving rats by microdialysis. British Journal of Pharmacology, 121: 1171-1179. https://doi.org/10.1038/sj.bjp.0701231.
12. Ciubotaru AD, Leferman CE, Ignat BE, Knieling A, Salaru DL, Turliuc DM, Foia LG, Dima L, Minea B, Hritcu LD, Cioroiu BI, Stoica L, Ciureanu IA, Ciobica AS, Stoica BA, Ghiciuc CM. Anti-Epileptic Activity of Mitocurcumin in a Zebrafish-Pentylenetetrazole (PTZ) Epilepsy Model. Pharmaceuticals (Basel). 2024 Nov 29;17(12):1611. doi: 10.3390/ph17121611. PMID: 39770453; PMCID: PMC11678555.
13. Ramalingam P., Ko Y.T. A Validated LC-MS/MS Method for Quantitative Analysis of Curcumin in Mouse Plasma and Brain Tissue and Its Application in Pharmacokinetic and Brain Distribution Studies. J. Chromatogr. B Analyt Technol. Biomed. Life Sci. 2014;969:101–108. doi: 10.1016/j.jchromb.2014.08.009.
14. Kunati S.R., Yang S., William B.M., Xu Y. An LC-MS/MS Method for Simultaneous Determination of Curcumin, Curcumin Glucuronide and Curcumin Sulfate in a Phase II Clinical Trial. J. Pharm. Biomed. Anal. 2018;156:189–198. doi: 10.1016/j.jpba.2018.04.034.