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Research Article

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Anticonvulsant Activity of Lacidipine against Mes- and Ptz-Induced Seizures

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Abstract: Epilepsy is one of the challenging medical conditions affecting neonates, children and elderly. It has affected 1710 to 9780/million populations within India. Although the report of this condition dates to the prehistoric era, drugs for its treatment are not 100% effective and more often lead to toxic effects. Therefore, a potent drug or a drug adjunct is the need-of-the-hour for providing effective control for this neurological condition. The aim of the present study is to evaluate lacidipine, a calcium channel blocker as a protective agent in seizures induced by standard models of epilepsy, namely maximal electroshock seizure model (MES) and Pentylenetetrazole (PTZ). Standard methods for the induction of seizures such as MES and PTZ were performed on male Wistar albino mice. The mice were then treated with vehicle/standard drug (sodium valproate)/ lacidipine. Further, the standard parameters of recovery duration were evaluated. The study revealed that lacidipine at a dose of 3mg/kg body weight significantly reduced the duration of hind limb tonic extension and postictal depression in MES. PTZ-induced seizures showed significantly reduced mean duration of hind limb tonic flexion, hind limb tonic extension, clonus and postictal depression. The findings from the present study suggest that lacidipine has potent anticonvulsant ability in better protection against MES-induced seizures over that of the PTZ-induced one.

Keywords: Lacidipine, epilepsy, maximal electroshock seizure model (MES), pentylenetetrazole (PTZ).

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1. INTRODUCTION

The word epilepsy has been derived from Greek, which means "to seize". This is constitutive of a set of neurological disorders persistent for an extended period characterized by seizures varying in duration [1]. Physiologically, this condition is characterized by an epileptic discharge from the cerebral neurons leading to sensory, motor, or autonomic phenomena, which may further lead to loss of consciousness [2]. A systematic meta-analysis has revealed that incidence of epilepsy was 61.4 per 100,000 persons annually, with greater prevalence in the low-income countries [3,4]. Neonates, children, and the elderly are at the highest risk of developing the disorder, with males having a greater susceptibility than females [5]. Although epilepsy as a condition was recognized in the prehistoric era, advances in its etiology and treatment have been seeing an upward trend annually [6]. Yet, treatments in the past have relied on superstitious and religious beliefs employing trephining, cupping and herbal medicines [7]. Some of the medications used previously were withdrawn owing to their adverse effects [8]. Some of the drugs fail to show efficacy leading to intolerable toxicity for the drugs, impaired memory function and depression in patients along with co-morbidity in refractory patients [9]. Owing to the high susceptibility rates, identification of a potent anti-epileptic drug has been in demand in the healthcare industry. Furthermore, recently available drugs fail to address the refractory epilepsy, which has risen to be a major hurdle in its treatment. Studies have proved that extracellular calcium influx triggers the epileptic activity owing to the paroxysmal depolarization shift (PDS). Therefore, therapies that focus on the inhibition of this calcium influx in turn alter the PDS, thereby reverting seizure spread [10]. A therapy entailing conventional anti-epileptic drugs along with a calcium influx modulator has proven effective on gaining control over seizure as well as the other characteristics of epilepsy [11]. Recent studies have demonstrated the use of dihydropyridine class of molecules in blocking calcium channels and therefore a potent adjuvant with the conventional therapy for treating seizures [12,13]. In this regard, the L-type calcium channels have proven to be a prominent target and blocking this resulted in effective control of epileptic seizures. The L- type calcium channel antagonists lead to the blockage of spontaneous influx of calcium and γ-amino butyric acid (GABA)-induced depolarization in HH tissues causing significant antiepileptic activity. With this background, the present study aims to evaluate the antiepileptic efficacy of lacidipine, a L-type calcium channel blocker, in prevention of experimentally induced seizures.

2. MATERIALS AND METHODS

2.1. Chemicals

Sodium valproate (Standard drug) and lacidipine were obtained from Sun Pharmaceuticals. Sodium valproate was prepared by dissolving 400 mg in 10 ml propylene glycol and lacidipine was dissolved in water to get a concentration of 3 mg/kg body weight. Freshly prepared sodium valproate was administered at 40 mg/kg body weight orally to the experimental animals. Pentylenetetrazole was obtained from Sigma Aldrich, Bangalore and prepared by dissolving in distilled water. This was administered at 70 mg/kg body weight intraperitoneally.

2.2. Animals

36 healthy wistar albino mice weighing 20-25 grams were selected from the central animal facility, JSS Medical College, Mysore. The experiments were approved by Institutional Animal Ethics Committee (JSSMC/IAEC/04/December – 2014) and conducted as per the guidelines of CPCSEA, Chennai, India. The animals were given food & water *ad libitum* both being withdrawn only a while prior to the experimentation. The experimental procedures were carried out after 24 hours to avoid any possible "kindling" effect. All the preparations except PTZ were administered orally whereas PTZ was administered intraperitoneally. The above test animals were divided into two groups such that one group was subjected to electroshock (50 mA intensity for 0.2 seconds) using auricular electrodes whereas the other group was subjected to chemoshock using PTZ. Only those mice showing the convulsive responses were used for the experiment & divided into 3X3 groups of six animals each for both the experiments (electroshock and chemoshock).

2.3. Experimental method

2.3.1. Maximal Electroshock Seizure Model (MES)

The procedure followed was according to Woodbury & Davenport (1955) [14]. Adult albino mice (20-25gms) were grouped into six animals per drug dose/vehicle. The administration and grouping were as per Table 1. Several factors are known to alter seizure susceptibility such as endocrine, nutritional, temperature and therefore male animals were preferred for the experiment to reduce variability. The groups were administered with the respective drug/ vehicle orally. 30 min after the treatment, the animals were subjected to stimulation using trans auricular electrodes at a current strength 150 mA for 0.2 s. The various phases of epilepsy parameters mentioned previously were recorded. Protection percentage was evaluated by "abolition of hind leg tonic extension (or) extension not >90°" (Fig 1). The parameters studied in MES method are: Tonic hindlimb flexion; Tonic hindlimb extension; Clonus; Postictal depression [from the regainment of consciousness to animals could walk away].

2.3.2. Chemical Model

Threshold PTZ seizures in rats & mice have long been considered as a model for the absence seizures because of its selective therapeutic response to drugs for absence seizures. In this study, PTZ at a dose of 75 mg/kg i.p. (dissolved in distilled water) was used to produce excitement, myoclonic jerks and clonic seizures. An occasional fatal tonic seizure may also occur. Seizures are produced in at least 97% of the animals. PTZ is given 30 minutes after the test drug. The PTZ response occurs about 5 to 10 minutes after administration. The mice are observed for half an hour post drug. Prolongation of duration of seizure latency was taken as an index of protection & indicates anticonvulsant activity of the test compound [15]. The parameters studied in PTZ method are: Seizure latency (time taken for onset of seizure); Myoclonic Jerk duration; Clonus; Straub's tail.

3. STATISTICAL ANALYSIS

Results were presented as Mean ± SEM. One-way analysis of

variance (ANOVA) followed by Duncan's multiple range test using SPSS Software (version 21.0, Chicago, USA) was used for multiple comparisons. For all the tests a 'p' value of 0.05 or less was considered for statistical significance [16].

4. RESULTS

In this study, the anticonvulsant activity of Lacidipine against MES- and PTZ-induced convulsions was evaluated. The parameters evaluated in the MES model showed (Table 2) that the mean duration of tonic hindlimb flexion (THLF) in the control group was 8.35 s whereas that of the standard drug, sodium valproate, reduced to 3.9 s. lacidipine reduced this duration to 5.63 s. Similarly, the mean duration of tonic hindlimb extension (THLE) in the control group was 9.11 s, that for sodium valproate was 1.81 s and lacidipine reduced it to 3.7 s. Likewise, the mean duration of clonus in the control group was 18.45 s, standard drug reduced it to 11.5 s whereas lacidipine showed a reduction to 12.2 s. Further, the mean duration of postictal depression in the control group was 93.17 s, which was reduced by sodium valproate to 13.33 s and by lacidipine to 14.6s. Overall, this study revealed that lacidipine provided 51.3% protection from MES-induced convulsions as against that of the standard drug (Table 2). Similarly, the parameters evaluated for PTZ- induced convulsions demonstrated (Table 3) that the mean duration of seizure latency in the control group was 26.3 s which was then increased to 85.5 s by sodium valproate and to 46.3 s by lacidipine. In addition, the mean duration of myoclonic jerks in the control group was 4.7 s, 1.65 s by the administration of the standard drug, sodium valproate and lowered to 1.95 s using lacidipine treatment. Similarly, the evaluation of mean duration of generalized clonic seizures in the control group was 10.83 s, which was reduced to 7.78 s by sodium valproate and to 9.6 s by the administration of lacidipine. The postictal depression duration that was observed for 94.1 s reduced to 15.2 s using sodium valproate and to 45.2 s using lacidipine. Overall, from the study it was noted that lacidipine provided 45.8% protection against PTZ-induced convulsions (Table 3).

Table 1: Summary of materials used in different types of screening methods

Group	Treatment	Details	
		Drug received	Type of experiment
MES-I	Control	Distilled water	MES
MES-II	Standard	Sodium valproate (40 mg/kg)	MES
MES-III	Test group I	Lacidipine 3mg/kg body weight	MES
PTZ-I	Control	Distilled water	PTZ
PTZ-II	Standard	Sodium valproate (40 mg/kg)	PTZ
PTZ-III	Test group I	Lacidipine 3mg/kg body weight	PTZ

Table 2: Effect of lacidipine on MES-induced seizures in Wistar albino mice

Group	Treatment	Drugs Received	THLF(sec)	THLE(sec)	Clonus (sec)	Postictal depression(sec)	Protection (%)
MES-I	Control	Distilled water	8.35 ± 1.79^{c}	9.11 ± 0.75^{c}	18.45 ± 2.02^{b}	93.17 ± 3.13^{b}	-
MES-II	Standard	Sodium valproate (40 mg/kg)	3.90 ± 0.57^{a}	1.81 ± 3.81^{a}	11.55 ± 0.91^{a}	13.33 ± 3.46^{a}	100
MES-III	Test group I	Lacidipine (3mg/kg)	5.63 ± 2.75^{b}	3.71 ± 0.86^{b}	12.27 ± 4.80^{a}	14.67 ± 3.46^{a}	51.30

Values are expressed as mean ± SEM. Means in the same column with distinct superscripts are significantly different (p≤0.05) as separated by Duncan's multiple range test. MES: Maximal electroshock.

Table 3: Effect of lacidipine on PTZ-induced seizures in Wistar albino mice

Group	Treatment	Drugs Received	Seizure latency(sec)	Myoclonic jerks(sec)	Clonic seizures (sec)	Postictal depression (sec)	Protection (%)
PTZ-I	Control	Distilled water	26.33 ± 0.64^{a}	4.70 ± 1.47^{b}	10.83 ± 0.67^{c}	94.10 ± 0.68^{c}	-
PTZ-II	Standard	Sodium valproate (40 mg/kg)	85.07 ± 2.34^{c}	1.65 ± 0.43^{a}	7.78 ± 1.05^{a}	15.20 ± 0.24^{a}	100
PTZ-III	Test group I	Lacidipine (3mg/kg)	46.32 ± 0.51^{b}	1.95 ± 1.11^{a}	9.62 ± 2.08^{b}	45.20 ± 0.95^{b}	45.81

Values are expressed as mean ± SEM. Means in the same column with distinct superscripts are significantly different (p≤0.05) as separated by Duncan's multiple range test.PTZ: Pentylenetetrazole.

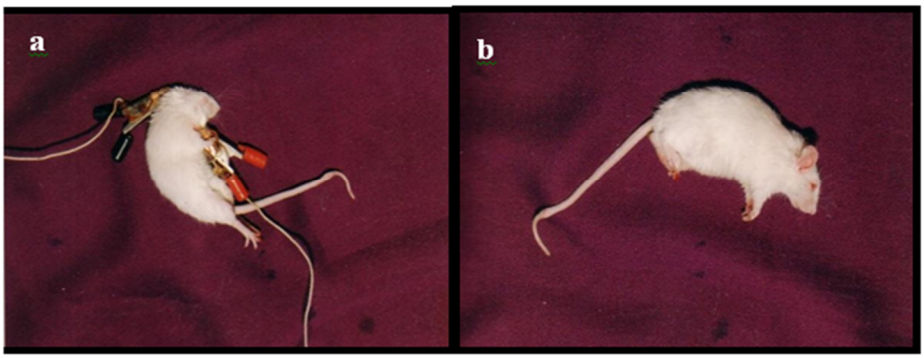

Fig 1: Maximal electroshock (MES) model showing lacidipine-induced tonic hind limb extension phase(a) and clonus phase(b) in Wistar albino mice

5. DISCUSSION

The present study is the first in evaluating the anti-epileptic activity of lacidipine, a calcium channel blocker on MES and PTZ-induced convulsions. Anticonvulsant drugs currently available can control over 70% of cases, whereas a smaller population demonstrates intolerance to these drugs [17]. Therefore, newer drugs were always on the lookout to optimize treatment methods for epilepsy. GABA agonists and calcium blockers have been studied for their beneficiary effects on the PTZ-induced convulsions and therefore in the present study, lacidipine, a calcium channel blocker, was used against the two standard models of epilepsy namely, MES and PTZ. Studies have proven the efficiency of both the models as standards to evaluate anti-epileptic activity with little difference among the results although PTZ describes the human exposures better than the MES model [18]. Yet, while evaluating drugs both the models provide conclusive evidences and hence used in the present study. In our study, lacidipine at 3 mg/kg body weight administered showed a remarkable reduction in the phases of convulsion parameters when compared to that of the control group. The results from our study revealed that lacidipine has significantly decreased THLE phase as well as extensor/flexion ratio in MES model as compared to that of the control group. Similarly, it also extended the onset of myoclonic jerks, generalized clonic seizure, tonic generalized extensor phase by significantly decreasing the duration of generalized clonic seizure and postictal depression phase induced by PTZ, as compared to that of the control. Also, it was noted that lacidipine provided 51.3% protection against the seizures induced in the MES model and 45.8% protection against that of PTZ model when compared with the standard drug sodium valproate. The THLE and THLF have been used as a primary end point for the assessment of anti-epileptic activity of several drugs in generalized tonic-clonic seizures [19, 20]. In a study by Kulkarni and George (1996) [21], the clonic seizures for less than 5 s were considered protective against the MES-induced seizures. Likewise, in our study it was reduced relative to that of the control group suggesting its protective ability. All the mice recovered following the MES test. However, it was also noted that a few mice in the PTZ model died after exhibiting various phases of convulsion. Mortality may be probably due to a combination of factors which include marked depression of vital medullary centers by persistent high concentration of circulating PTZ, hypoxia and exhaustion resulting from initial and recurrent seizures. Similar results were obtained by several studies suggesting a chronic toxicity exerted by PTZ at a persistently high dosage [22,23]. Control group animals (which received propylene glycol) were not protected against both MES & PTZ models. The potential benefits of either competitive or non-competitive antagonists of calcium channels is not clear in bringing about antiseizure effects, yet competitive antagonists are expected to revert the antagonistic effect by the increased concentration of the agonist. It is suggested in several studies that a noncompetitive antagonist is more potent over the competitive one as the effects may be retained even at an increasing concentration of the agonist[24,25]. However, findings from our study suggest that although lacidipine is a competitive antagonist it has the capability to act as a potent agent in bringing down the seizures induced by MES as well as PTZ. Yet, its use as an adjunct with the standard drugs to reduce the toxic effects of the drugs and in turn reduces the seizure incidences. Furthermore, studies have suggested that the drugs that often present positive effects in the MES model by exerting activity like that of the phenytoin by affecting the sodium gated channels [26]. Similarly, the drugs that prove positive on the PTZ-induced seizures exert their action through the GABAergic brain receptors or altering post-synaptic receptors for GABA. In addition, these drugs may also act on the calcium channels by blocking them thereby inhibiting the influx of calcium ions into the cells [27]. In a study previously, several calcium channel blockers were evaluated for their anticonvulsant potential. It was expected to act by mobilization of calcium ions from intracellular stores thereby bringing about anticonvulsant activity[28]. This was one of the preliminary studies on anticonvulsant activity of dihydropyridine class of molecules. Likewise, in our study the action of lacidipine revealed that the compound is effective in reducing the seizures by a combination of the above-said mechanisms suggesting the promising role of lacidipine as a potent anti-epileptic drug.

6. CONCLUSION

Lacidipine, a calcium channel blocker is known to block the influx of calcium ions by a competitive mechanism. In this study, potential ability of lacidipine as an anti-epileptic agent was evaluated. The results suggested that the drug indeed provided protection against the MES- and PTZ-induced seizures, wherein it was more effective on the MES model than PTZ model. Our findings form the basis for further studies in understanding the mechanism of lacidipine action in bringing about protection against seizures during epilepsy conditions. Further studies on various models of epilepsy need to be performed in order to affirm the activity and extrapolate the same on human subjects.

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8. AUTHORS CONTRIBUTION STATEMENT

Concept and design: Pushpa V H; Data acquisition**:** Jayanthi M K and Mallikarjun S; Critical revision of manuscript: Ramith Ramu. The manuscript was completed with equal contributions from all the authors in terms of preparing the manuscript.

9. CONFLICT OF INTEREST

Conflict of interest declared none.

10. REFERENCES

1. Baradaran Rahimi VB, Askari VR, Hosseini M, Yousefsani BS, Sadeghnia HR. Anticonvulsant Activity of Viola tricolor against Seizures Induced by Pentylenetetrazol and Maximal Electroshock in Mice. Iran J Med Sci. 2019;44(3):220-6. PMID 31182888.

2. Levinson S, Tran CH, Barry J, Viker B, Levine MS, Vinters HV, Mathern GW, Cepeda C. Paroxysmal discharges in tissue slices from pediatric epilepsy surgery patients: critical role of GABAB receptors in the generation of ictal activity. Front Cell Neurosci.

2020;14:54. doi: 10.3389/fncel.2020.00054. PMID 32265658.
3. Beghi E. The epidemiology of epilepsy. Neuroepidemiology. 2020;54(2):185-91. doi: 10.1159/000503831, PMID 31852003.
4. Beghi E, Hesdorffer D. Prevalence of epilepsy – an unknown quantity. Epilepsia. 2014;55(7):963-7. doi: 10.1111/epi.12579, PMID 24966095.
5. Samba Reddy D. Sex differences in the anticonvulsant activity of neurosteroids. J Neurosci Res. 2017;95(1-2):661-70. doi: 10.1002/jnr.23853, PMID 27870400.
6. Chaudhary UJ, Duncan JS, Lemieux L. A dialogue with historical concepts of epilepsy from the Babylonians to Hughlings Jackson: persistent beliefs. Epilepsy Behav. 2011;21(2):109-14.doi: 10.1016/j.yebeh.2011.03.029.
7. Peterson SL, Albertson TE, editors. Neuropharmacology methods in epilepsy research. Boca Raton: CRC press Press; 2019. p. 1-269.
8. Korinthenberg R, Burkart P, Woelfle C, Moenting JS, Ernst JP. Pharmacology, efficacy, and tolerability of potassium bromide in childhood epilepsy. J Child Neurol. 2007;22(4):414-8. doi: 10.1177/0883073807302758, PMID 17621520.
9. Luoni C, Bisulli F, Canevini MP, De Sarro G, Fattore C, Galimberti CA, Gatti G, La Neve A, Muscas G, Specchio LM, Striano S, Perucca E, SOPHIE Study Group. Determinants of health-related quality of life in pharmacoresistant epilepsy: results from a large multicenter study of consecutively enrolled patients using validated quantitative assessments. Epilepsia. 2011;52(12):2181-91. doi: 10.1111/j.1528-1167.2011.03325.x, PMID 22136077.
10. Owoalade SA, Martin Lou DM, Yadav K, Poudel S. A Review on anti-epileptic activity of seaweed Ecklonia cava. J Drug Delivery Ther;9(1-s):425-32. doi: 10.22270/jddt.v9i1-s.2335.
11. Wormuth C, Lundt A, Henseler C, Müller R, Broich K, Papazoglou A, Weiergräber M. Review: Cav2.3 R-type Voltage-Gated Ca2+ Channels - Functional Implications in Convulsive and Non-convulsive Seizure Activity. Open Neurol J. 2016;10:99-126. doi: 10.2174/1874205X01610010099, PMID 27843503.
12. Uppin V, Aa N, Bharatha A. Antiepileptic effect of calcium channel blockers alone and in combination with phenytoin on MES induced epilepsy in rats. IJCBR. 2017;3(3). doi: 10.5455/ijcbr.2017.33.04.
13. Jain R, Bankey N, Jain A. Anticonvulsive effects of nifedipine and flunarizine on maximal electroshock seizures induced seizures. Natl J Physiol Pharm Pharmacol. 2018;8(9):1317-20. doi: 10.5455/njppp.2018.8.0519029052018.
14. Woodbury DM, Naisbitt MS. Effect of diphenylhydantoin on electrolytes and radiosodium turnover in brain and other tissues of normal, hyponatremic and postictal rats. J Pharmacol Exp Ther. 1955;115(1):74-95.
15. Dhir A. Pentylenetetrazol (PTZ) kindling model of epilepsy. Curr Protoc Neurosci. 2012; Chapter(9): Unit 9. 37. doi: 10.1002/0471142301.ns0937s58.
16. Ramu R, Shirahatti PS, Zameer F, Ranganatha LV, Nagendra Prasad MN. Inhibitory effect of banana (Musa sp. var. Nanjangud rasa bale) flower extract and its constituents Umbelliferone and Lupeol on α-glucosidase, aldose reductase and glycation at multiple stages. South African Journal of Botany. 2014;95:54-63. doi: 10.1016/j.sajb.2014.08.001.
17. Sayyah M, Mandgary A, Kamalinejad M. Evaluation of the anticonvulsant activity of the seed acetone extract of Ferula gummosa Boiss. against seizures induced by pentylenetetrazole and electroconvulsive shock in mice. J Ethnopharmacol. 2002;82(2-3):105-9. doi: 10.1016/s0378-8741(02)00166-6, PMID 12241984.
18. Mombeini T, Asadpour Behzadi BA, Ejtemaei R, Tahmasbi F, Kamalinejad M, Dehpour AR. Anticonvulsant effect of Alcea aucheri on pentylenetetrazole and maximal electroshock seizures in mice. Basic Clin Neurosci. 2020;11(3):369-77. doi: 10.32598/bcn.11.2.2064.1, PMID 32963729.
19. Krall RL, Penry JK, White BG, Kupferberg HJ, Swinyard EA. Antiepileptic drug development: II. Anticonvulsant drug screening. Epilepsia. 1978; 19(4): 409-28. doi: 10.1111/j.1528-1157.1978.tb04507.x.
20. Rao VS, Rao A, Karanth KS. Anticonvulsant and neurotoxicity profile of Nardostachysjatamansi in rats. J Ethnopharmacol. 2005;102(3):351-6. doi: 10.1016/j.jep.2005.06.031, PMID 16095854.
21. Kulkarni SK, George B. Anticonvulsant Action ofWithania somnifera (Aswaganda) Root Extract against Pentylenetetrazol-induced Kindling in Mice. Phytother Res. 1996;10(5):447-9. doi: 10.1002/(SICI)1099-1573(199608)10:5<447::AID-PTR869>3.0.CO;2-M.
22. Raju S, Pl B, Hn N, Shanbhag A. A study on the anticonvulsant activity of Withania Somnifera (Dunal) in albino rats. Natl J Physiol Pharm Pharmacol. 2017; 7(1):17. doi: 10.5455/njppp.2016.6.06151120072016.
23. Purushotham K. Evaluation of anticonvulsant activity of ethanolic extract of Nardostachys jatamansi roots in albino rats [doctoral dissertation]. KRN, India: Rajiv Gandhi University of Health Sciences; 2012.
24. Yamaguchi S, Donevan SD, Rogawski MA. Anticonvulsant activity of AMPA/kainate antagonists: comparison of GYKI 52466 and NBQX in maximal electroshock and chemoconvulsant seizure models. Epilepsy Res. 1993;15(3):179-84. doi: 10.1016/0920-1211(93)90054-B.
25. Fritsch B, Stott JJ, Joelle Donofrio J, Rogawski MA. Treatment of early and late kainic acid-induced status epilepticus with the noncompetitive AMPA receptor antagonist GYKI 52466. Epilepsia. 2010; 51(1):108-17. doi: 10.1111/j.1528-1167.2009.02205.x.
26. Rogawski MA. Molecular targets versus models for new antiepileptic drug discovery. Epilepsy Res. 2006;68(1):22-8.doi: 10.1016/j.eplepsyres.2005.09.012.
27. Jung ME, Lal H, Gatch MB. The discriminative stimulus effects of pentylenetetrazol as a model of anxiety: recent developments. Neurosci Biobehav Rev. 2002;26(4):429-39. doi: 10.1016/S0149-7634(02)00010-6.
28. De Sarro G, De Sarro A, Federico F, Meldrum BS. Anticonvulsant properties of some calcium antagonists on sound-induced seizures in genetically epilepsy prone rats. General Pharmacology: The Vascular System. 1990;21(5):769-78. doi: 10.1016/0306-3623(90)91032-M.