

## ANTIEPILEPTIC EFFECT OF CALCIUM CHANNEL BLOCKERS ALONE AND IN COMBINATION WITH PHENYTOIN ON MES INDUCED EPILEPSY IN RATS

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### ABSTRACT

**Background:** The currently available antiepileptic drugs have a low therapeutic index, and provide satisfactory seizure control in only 60-70% of patients. Calcium channel blocker has shown potentials of a useful add-on drug for the existing antiepileptic drugs. **Objectives:** To study the effect of calcium channel blockers (CCBs) nifedipine and verapamil on maximal electroshock (MES)-induced convulsions and also to evaluate their effect in combination with Phenytoin. **Methods:** For this study, Male Wistar rats were used. Effects of nifedipine (5 mg/kg), verapamil (20mg/kg) alone and in combination with phenytoin (25mg/kg) were studied in MES model. Abolition of hind limb tonic extension and reduction of duration of tonic extension was considered as the index for antiepileptic activity in MES. **Results:** Verapamil and nifedipine produced significant antiepileptic effect alone. Verapamil and nifedipine potentiated the antiepileptic effect of phenytoin sodium. **Conclusion:** Dose of phenytoin sodium can be reduced in epileptic patients receiving verapamil or nifedipine for some other clinical conditions. Verapamil and nifedipine can also be used alone in the treatment of generalized tonic clonic seizures. However it needs further confirmation to establish clinical utility of calcium channel blockers.

**KEY WORDS:** Nifedipine, verapamil, phenytoin, MES seizure rats.

### INTRODUCTION

Epilepsy a chronic disorder of heterogeneous symptoms characterized by recurrent seizures, seizures are finite episodes of brain dysfunction resulting from abnormal discharge of cerebral neurons. The causes of seizures are extremely diverse and include the full range of neurological diseases from infections to neoplasm, head injury. Heredity has proved to be a predominant factor in some subgroups [1,2].

Approximately 1% of the world's population has epilepsy, the second most common neurological disorder after stroke. The therapeutic objective of the treatment of epilepsy is complete seizure control without excessive side effects. Uncontrolled epilepsy can result in neuropsychiatric and social impairment, lower quality of life, greater morbidity and higher risk of death [1-3]. Several different classes of drugs are available for treatment of the epilepsy. They act by different mechanisms, such as blockade of SRF(Sustained repetitive firing) by enhancing sodium channel inactivation, enhancing GABAergic inhibition or by reducing T- calcium currents, enhancing K<sup>+</sup> current blockade of NMDA(N-methyl-D-aspartate) receptors, blockade of AMPA (aminomethylphosphonic acid) receptors and blockade of Kainic acid receptors [3]. These drugs have limitations mainly due to their toxicity profile and drug-drug interactions. In addition, epilepsy remains resistant to drug therapy in about one third of patients, thus encouraging the development of drugs that act on mechanisms underlying pharmacoresistance.

The role of inflammation and immune mediated insults in seizure initiation and process of epileptogenesis has been supported by many studies [4, 5]. Experimental studies in animals have proved that neuroinflammation can lead increase in permeability of blood brain barrier and excitability of nervous tissue [6,7].

Role of oxidative stress in epileptogenesis process has been supported in various experimental studies [8]. Mitochondrial related oxidative stress plays an important role in the epileptogenesis in lithium- pilocarpine model of temporal lobe epilepsy in animals [9]. Anti-oxidants can reduce the severity of neuronal injury caused by oxidative stress and therefore development of seizures in some animal models [10].

Clinically used calcium channel blockers act by blocking L-type and T-type of calcium channels which are present in various tissues including brain and they are commonly prescribed for treatment of various clinical conditions such as hypertension, angina and cardiac arrhythmias [11]. In various experimental animal models, calcium



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channel blockers have been shown to exhibit the anti-inflammatory and antioxidant properties [12]. In addition calcium channel blockers have several advantages over the existing anti-epileptic drugs, such as no effect on hepatic microsomal enzymes, devoid of sedation, interference with quality of life and wide therapeutic range. They are even best suited for elderly individuals.

The present study is therefore undertaken to evaluate the antiepileptic effect of clinically used calcium channel blockers and also to evaluate their ability to potentiate the antiepileptic effect of existing drugs.

#### Objectives:

1. To evaluate the antiepileptic potential of CCBs, phenytoin and comparison with phenytoin.
2. To evaluate the ability of the CCBs to potentiate the anti-epileptic effect of phenytoin.

### MATERIALS AND METHODS

**Study design:** Randomized, an experimental study

**Ethics approval:** The study was approved by institutional ethics committee

**Study locus and period:** Study was conducted in department of Pharmacology, Shri B M Patil Medical College, Bijapur

**Animals:** Inbred, 36 male wistar rats, weighing 150-200g were selected for the present study.

**Maximum electric shock method (MES induced epilepsy):** The above animals were subjected to electroshock of 150 mA intensity for 0.2 seconds through auricular electrodes, [covered in cotton wool & saline moistened] [12].

**Inclusion criteria:** A majority of rats showed tonic flexion of fore & hind limbs with tail erection, tonic extension of both fore & hind limbs, clonus, stupor followed by post ictal depression & recovery. Only those rats showing the convulsive responses were used for experiment.

**Grouping of animals:** Rats were divided into 6 groups and in each group n=6

**Group 1:** Vehicle control (MES with our any treatment)

**Group 2:** MES and treated with standard drug (phenytoin 25mg/kg)

**Group 3:** MES and treated with Nifedipine (5mg/kg)

**Group 4:** MES and treated with Verapamil (20mg/kg)

**Group 5:** MES and treated Nifedipine (2.5mg/kg) + phenytoin (12.5mg/kg)

**Group 6:** MES and treated Verapamil (10mg/kg) + phenytoin (12.5mg/kg)

The above doses were selected from the pilot study and the combination were taken half of the doses as assuming to produces potentiation effect.

#### Parameters studied:

1. Presence or absence of Tonic Hind Limb Extension (THLE) in rats.
2. Duration of tonic extension.

No animals were sacrificed in the study. Animals were rehabilitated according to CPCSEA guidelines.

**Statistical analysis:** All the values were expressed as

the mean  $\pm$  SEM and ( $p < 0.05$ ). The latency to convulse and duration of HLE were analysed by one way analysis of variance (ANOVA). For comparison between multiple groups, one way ANOVA followed by post hoc Tukey's test was performed. Analysis of the seizure protection and percentage mortality was done by Fisher's exact test. The " $p$ " value of  $< 0.05$  was considered as statistically significant.

### RESULTS

In Maximal Electroshock (MES) Method the parameters like the latency to convulse, duration of tonic convulsion (hind limb tonic extension i.e HLE), the percentage seizure protection and the percentage mortality were recorded and results obtained in different groups represented in figures. As seen in Figure 1, Phenytoin group (Group II) shown complete abolition of convulsion, highly statistically significant decrease in duration of tonic hind limb extension, increase in seizure protection (83.33%) and any percentage mortality compared to control group ( $p < 0.05$ ).

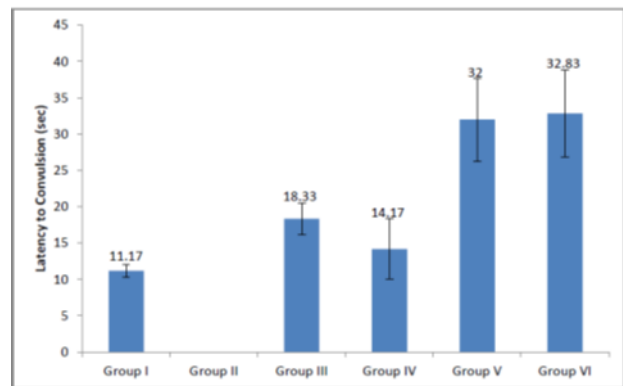


Figure 1. Latency to convulsions of experimental groups in MES method

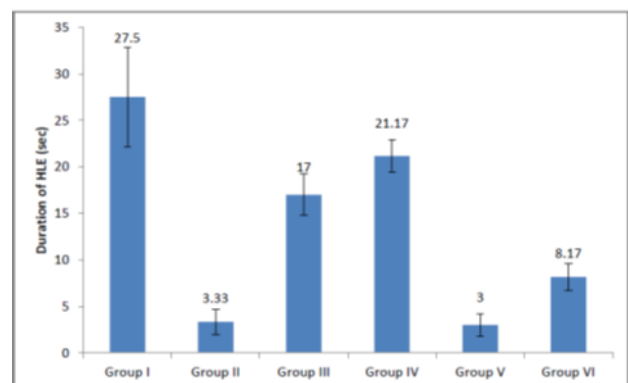
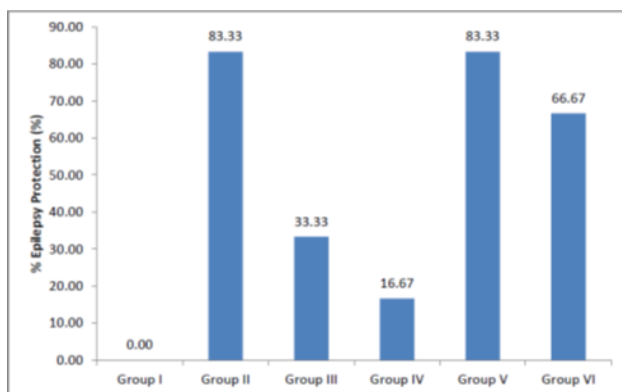


Figure 2. Duration of Hand limb extension (in sec)

In only calcium channel blockers (Nifedipine and Verapamil) groups, Nifedipine group (Group III) and Verapamil group (Group IV) shown statistically non significant increase in latency to convulse and decrease in duration of tonic hind limb extension phase (Figure 3) when compared to control group ( $p < 0.05$ ). Whereas, in both these group statistically non significant increased percentage epilepsy protection (Figure 2) and decreased percentage mortality was found when compared with control group (Group I).



**Figure 3. Percentage epilepsy protection of experimental groups in MES method**

Phenytoin and calcium channel blockers (Nifedipine and Verapamil) combination groups (Group V and VI) depicts statistically significant increase in latency to convulse and decrease in duration of HLE when compared to control group. In both these group statistically significant increased percentage epilepsy protection and decrease in percentage mortality was noted when compared to control group (Group I) ( $p < 0.05$ ).

## DISCUSSION

In our study finding showed, in only Nifedipine group (Group III) and Verapamil group (Group IV) have shown statistically non significant increase in latency to convulsion and decrease in duration of HLE when compared to control group (Group I). Whereas, in both these group statistically non significant increased percentage epilepsy protection and decreased percentage mortality was found when compared with control group (Group I). Two out of six animals were protected against MES induced seizures in all the phases with Nifedipine and only one out of six animals was protected against MES induced seizures in all the phases with Verapamil. Whereas, Sodium valproate and calcium channel blockers (Nifedipine and Verapamil) combination groups (Group V and VI) results depicts statistically significant increase in latency to convulsions and decrease in duration of clonic convulsions when compared to control group (Group I) ( $p < 0.05$ ). In both these group statistically significant increased percentage epilepsy protection and decrease in percentage mortality was noted when compared to control group (Group I). Five out of six animals were protected against MES induced seizures in all the phases with Nifedipine and similarly four out of six animals were protected against MES induced seizures in all the phases with Verapamil. Further, when we observed both these calcium channel blockers (Nifedipine and Verapamil) combination groups with Phenytoin and standard control group (Group II) treated with only Phenytoin showed almost same potential antiepileptic results on experimental animals.

Hadizadeh F et al [13] studied anticonvulsant activity of two novel 4 (1- (4-fluorobenzyl)-5 imidazolyl) dihydro-

pyridine derivatives in mice using pentylenetetrazole and electroshock seizure models. Sodium valproate, phenytoin sodium and nifedipine were used as positive controls. The above study demonstrated that the two dihydropyridine derivatives have significant anticonvulsant activity in both the animal experimental models.

Patten S R et al [14] evaluated new substituted dihydropyridine derivatives for their anticonvulsant activity in rats using Pentylenetetrazole, Strychnine and Maximum Electroshock models. His study confirmed significant anticonvulsant properties of these compounds.

Shafiee A et al [15] studied anticonvulsant activities of alkyl' arylalkyl and cycloalkyl ester analogues of nifedipine in pentylenetetrazole induced seizures in mice at Department of Medicinal Chemistry and Toxicology, Tehran University, Tehran. The study confirmed that all the above analogues of nifedipine had significant effect in reducing the latency and duration of seizures.

A study carried out by Umukoro S et al [16] to evaluate the anticonvulsant activity of two Calcium channel blockers, Verapamil and Nifedipine in Swiss albino mice. In this study, strychnine was administered intraperitoneally in the dose of 1mg/kg to induce convulsions. Both the calcium channel blockers prolonged the onset of seizures, when compared to controls ( $p < 0.05$ ).

Study done at university of Reggio Calabria [17], Italy showed that calcium channel blockers like flunarizine, nifedipine, nicardipine, nimodipine, nitrendipine and diltiazem exhibited significant anticonvulsant activity in DBA/2 mice. In this study the convulsions were induced in experimental animals by auditory stimulation (109 db). However, verapamil did not exhibit anticonvulsant activity in this study.

Sathyanarayan Rao K et al [18] carried out study to evaluate anticonvulsant activity of Amlodipine in mice using MES and PTZ models. Sodium valproate and normal saline were used as standard and control respectively. This study indicated that Amlodipine may be considered as add-on therapy for epilepsy.

Shitak R et al [19] carried out a study to evaluate anti-seizure efficacy of Nimodipine in pentylenetetrazole and kainic acid combined seizure models in mice. Nimodipine (5mg/kg, ip) significantly protected mice from convulsions in both of the combination *in vivo* seizure models.

Brahmane et al [20] evaluated anti-convulsant activity of cinnarazine and nifedipine in mice using maximal electroshock and pentylenetetrazole models and showed that both the calcium channel blockers had significant anticonvulsant activity as compared to controls. It was also observed that both the calcium channel blockers significantly potentiated the effect of sodium valproate in both the models.

## CONCLUSION

Verapamil and nifedipine produced significant antiepileptic effect alone. Verapamil and nifedipine potentiated the antiepileptic effect of phenytoin sodium. Dose of phenytoin sodium can be reduced in epileptic patients receiving verapamil or nifedipine for some other clinical

conditions. Verapamil and nifedipine can also be used alone in the treatment of generalized tonic-clonic seizures. However, it needs further confirmation to establish clinical utility of calcium channel blockers.

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**Conflicts of Interest:** There are no conflicts of interest.

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