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# Comparison of the Anticonvulsant Effect of Flunarizine, Nimodipine and Sodium Valproate in Rats

V.M. Thorat and Jaydeep M. Bairagi
*Department of Pharmacology, Krishna Institute of Medical Sciences, 415110 Karad, Maharashtra, India*

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**Corresponding Author:**
V.M. Thorat
*Department of Pharmacology, Krishna Institute of Medical Sciences, 415110 Karad, Maharashtra, India*

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**Abstract:** The calcium channel blockers flunarizine, comparing the inhibitory effects of nifedipine and their efficacy with sodium valproate are induced in MES and PTZ in broad-spectrum anticonvulsant Alzheimer's mice. Calcium ion and its entry play an important role in origin of seizures. The objective of the study is to investigate effect of cerebro selective Calcium Channel Blockers (CCBs), flunarizine and nimodipine with three different dosages and sodium valproate with one dose on MES (Maximal Electroshock Seizures) and PTZ (Pentylenetetrazol) induced convulsions in wistar rats. The CCBs have established themselves as very effective therapeutic agents in various disorders of the cardiovascular system like systemic hypertension, supraventricular arrhythmias, angina pectoris, etc., in central nervous system like migraine, vertigo, subarachnoid hemorrhage, etc. and for reversal of resistance in chemotherapy. In this study, the pre-clinical evaluation for anticonvulsant profile of cerebro selective CCBs viz. Flunarizine and Nimodipine and their comparison with standard drug sodium valproate using conventional models of experimental epilepsy, i.e. by MES and PTZ induced seizures in wistar rats.

## INTRODUCTION

Epilepsy has been recognized since antiquity. The word epilepsy derives from the Greek verb 'epilambanein', meaning to be seized, to be overwhelmed by surprise' or take hold of, indicates that the person detained is possessed or at least out of reach. Epilepsy is the most prevalent chronic brain condition but is still overshadowed by ignorance and belief that can only be resolved through massive difficulties. The clinical features of epilepsy often have provoked fear and early treatment ranged from exorcism to bloodletting (Trescher and Lesser, 2008). Thus, there are sites of Calcium Channel Blockers (CCBs) that enable them to cross the Blood Brain Barrier (BBB) and gives an important evidence for the presence of their central effects (McDevitt *et al*., 1991; Godfraind *et al*., 1986). Ethosuximide is a major drug used for the treatment of absence seizures which acts by inhibiting T type of calcium channel and sodium valproate too acts by weak attenuation of calcium mediated T current (Tripathi, 2008). Keeping this in view, effect of potent CCBs (flunarizine and nimodipine) with three different dosages and sodium valproate with one dose was studied using

conventional models of experimental epilepsy in animals, i.e. by inducing Maximal Electro Shock (MES) and Pentylenetetrazole (PTZ) seizures in wistar rats.

**Aims and objectives:** To investigate effect of cerebro selective Calcium Channel Blockers (CCBs), flunarizine and nimodipine with three different dosages and sodium valproate with one dose on MES (Maximal Electroshock Seizures) and PTZ (Pentylenetetrazol) induced convulsions in wistar rats. To compare the anticonvulsant activity of cerebroselective CCBs flunarizine and nimodipine with sodium valproate.

**Literature review:** Some of history's well known personalities have been sufferers of this dreaded disease. Alexander the Great, Julius Caesar and Napoleon were a few commanders afflicted by epilepsy. Well known writers like Charles Dickens, Flaubert, Fyodor Dostoyevsky and Van Gogh, the well-known painter were victim of this ailment. Alfred Nobel and Richard Burton also suffered from this disease. A number of world class players like Jonty Rhodes and Tony Grieg are also suffering from this disease (Gupta, 2001). FDA approved felbamate and gabapentine in 1993, lamotrigine in 1994, topiramate in 1996 and tiagabine in 1997 (Burneo *et al*., 2005). Factors contribute to the higher incidence and prevalence of epilepsy in developing countries like limited access to health care compounds, the problems of birth injury and head trauma, poor sanitation leading high rates of infectious diseases of CNS and combination of local social perceptions, government policies and anti-epileptic drug availability (Burneo *et al*., 2005; De Bittencourt *et al*., 1996a, b; Preux and Druet-Cabanac, 2005). The incidence of epilepsy by age is bimodal in developed countries but is not as evident in developing countries (Trescher and Lesser, 2008; Forsgren *et al*., 2005). Rates are higher in first decade, particularly before the age of 1 year and then decline during childhood reaching minimum between 20-39 years of age with secondary rise in incidence after 60 years of age (Brodie and Kwan, 2005). The age specific incidence of epilepsy remains high throughout adulthood, largely related to remote symptomatic epilepsy occurring as a result of infection and trauma (De Bittencourt *et al*., 1996a, b). A study, sponsored by the UK government for international development states that India spends US $1.7 billion per year toward the care for epilepsy (Ashraf, 2002). Over the past decade, the treatment options for epilepsy have expanded dramatically but the basic principles of treatment are relatively unchanged. Some treatment failures can be attributed to misdiagnosis of non-epileptic paroxysmal events as seizures (Trescher and Lesser, 2008; Devinsky, 1999). It is important to begin by considering some broad principles that need to be applied to the treatment of an individual patient. The important points are as follows (Trescher and Lesser, 2008; Aminoff and Kerchner, 2013).

**Certainty of diagnosis:** The diagnosis of seizures or epilepsy should be secure. There is little or no place for therapeutic trail, when the diagnosis is uncertain.

**Deciding when to start treatment:** Initiating or changing anti-epileptic drug therapy needs full and adequate discussion with the patient who should be made fully aware of the aims of treatment, the benefit and adverse effects. Many decisions to be made in treatment of epilepsy are not clear cut and require balanced judgment. Purpose of treatment: The ultimate aim of treating epilepsy will be no seizure and no drugs. Unfortunately, this is not readily achievable for many patients with epilepsy. The first step is treating epilepsy with minimum effective dose of optimally effective anti-epileptic drugs. Monotherapy will be effective in 50-70% of new patients but polytherapy is necessary in more severe form of epilepsy.

**Choice of treatment:** In choosing between different drugs judgment must be addressed to relative efficacy, tolerability and its safety. Antiepileptic medications with simple schedule with no more than twice daily dosing and without regular blood monitoring levels should be preferred. Compliance is a major issue in long term treatment. Compliance can be improved by limiting to a minimum the number of daily doses.

**Discontinuation of therapy:** Long term anticonvulsant drugs are associated with increased potential for morbidity, therefore, the possibility of discontinuation of therapy should be balanced against the risk of anger of seizure recurrence. The importance of total discontinuation of therapy is not established. Abrupt discontinuation of anticonvulsant drugs is believed to be associated with increased seizures and Status Epilepticus. Most physicians agree that a tapered drug withdrawal is advisable perhaps over as long as 6 months. Pediatric neurologist generally favour 2 years of seizure control before attempting to withdrawn drugs.

**Ketogenic diet:** The ketogenic diet has been used in the treatment of epilepsy for almost one hundred years ago. An osteopath in early 20th century prior to introduction of anticonvulsant medication discovered that starving people with epilepsy of all but water led to remission of seizures. However such extremes treatment cannot be advised. This diet involves low carbohydrate and high fat intake with resultant ketosis. It is an effective alternative for drug resistant epilepsy, particularly in children.

**Endogenous antiseizure substance; Sharma and Sharma (2011):** Some sort of regulatory mechanism exist within the body otherwise why without outside intervention there should be spontaneous arrest of seizure activity after an attack and why the brain could remain seizure free for some time between two intervening attacks (post ictal refractory period). Elevation of seizure threshold by the test drug is taken as measure of its efficacy. Use of animals more than once a day is not recommended as post ictal rise in seizure threshold has been noted (Loscher and Schmidt, 1988). Kindling is done in rats and mice by giving electroshock via corneal electrodes. Mice is kindled by once daily application of 3 mA current of 60 Hz frequency for 2 sec while rats by twice daily application of 8 mA current of 60 Hz frequency for 4 sec via. corneal electrodes. Racine (1972) stage 5 seizures indicate that the animal is kindled. Putative antiepileptic drugs can be tested after the animals have had stage 5 seizures for 10 consecutive times (Racine, 1972; Sangdee *et al*., 1982; Matagne and Klitgaard, 1998). Kindling by stimulation of other brain areas: Kindled seizures can also be produced by stimulation of other brain areas like neocortex or hippocampus in rats. Development of Rapidly Recurring Hippocampal Seizures (RRHS) model of kindling in rats is briefly described as below (Lothman *et al*., 1985). Most of the animal models for screening of antiepileptic drugs are basically models of seizures rather than epilepsy, which is a condition of chronically recurrent spontaneous seizures. Genetic animal models more closely approximate human epilepsy and give opportunity to study genetic and biochemical basis of epilepsy (Mittal, 2009).

## MATERIALS AND METHODS

Albino Wistar rats of either sex (weighing 100-180 g), bred in the Central Animal House of the Krishna Institute of Medical Sciences, Karad were obtained. Total number of 120 animals were used in our study. Drugs used were flunarizine hydro-chloride, nimodipine and sodium valproate (all obtained from Okasa Pharmaceuticals) and pentylenetetrazol (Sigma-Aldrich Chemicals Pvt. Limited). Vehicle used were 5% Tween 80 (Loba Pharmaceuticals) and distilled water. The volume of injection was 2 mL $kg^{-1}$ for flunarizine, nimodipine, sodium valproate and pentylenetetrazol. All the test animals were screened for standard convulsive responses and subjected to further experiments of this study after a gap of 7 days to avoid any possible "kindling" effect. Colour coding with black, green, blue and red was done to identify the same animal before subject to Maximal Electroshock Seizures (MES) in rats (colour were applied to base of tail of animals).

## RESULTS AND DISCUSSION

All the animals used in different study groups for MES Model were screened before subjecting them to maximal electric seizure pattern and duration of THLF, THLE, clonus and stupor was measured in seconds. There was no significant difference in these study groups on different seizures parameters (THLF, THLE, clonus and stupor) before administration of respective dosages using one way ANOVA test ($p>0.05$).

There was no significant difference in mean duration of THLF of 5 and 10 mg $kg^{-1}$ flunarizine group when compared to it's before treatment values using paired t test ($p>0.05$). There was significant decrease in mean duration of THLF in 15 mg $kg^{-1}$ flunarizine group when compared to it's before treatment values using paired t test ($p<0.05$). There was significant difference in mean duration of THLF in distilled water, 5, 10 and 15 mg $kg^{-1}$ flunarizine groups using one way ANOVA test ($p<0.05$). Post hoc Tukey-Kramer multiple comparison test revealed that the mean duration of THLF in 15 mg $kg^{-1}$ flunarizine group was significantly less as compared to distilled water group ($p<0.05$). Flunarizine at 15 mg $kg^{-1}$ dose produced 33.33% protection against THLF in rats. Graphical representation for duration of THLF after treatment with flunarizine at different doses in MES Model is as shown in Table 1.

No animal was protected for THLF in 2.5, 5 and 10 mg $kg^{-1}$ nimodipine groups. Graphical representation for duration of THLF after treatment with nimodipine at different doses in MES Model. The results for duration THLE, before and after giving nimodipine at different doses are shown in Table 2.

There was extreme significant decrease in mean duration of stupor in 300 mg $kg^{-1}$ sodium valproate group when compared to its before treatment values paired t test ($p<0.001$). Percentage protection against stupor in rats at 300 mg $kg^{-1}$ sodium valproate group was 100%. The results for duration of stupor, before and after giving sodium valproate at dose of 300 mg $kg^{-1}$ and distilled water are shown in Table 3.

The mean latency period was significantly high in 300 mg $kg^{-1}$ sodium valproate group when compared with distilled water group using unpaired t test ($p<0.05$). The mean duration of PTZ induced seizures was extreme significantly less in 300 mg $kg^{-1}$ sodium valproate group when compared with distilled water group using unpaired t-test ($p<0.001$). Percentage protection against PTZ induced seizures in rats at 300 mg $kg^{-1}$ sodium valproate group was 33.33%. The results for latency period and duration of PTZ induced seizures in 300 mg $kg^{-1}$ sodium valproate and its control (distilled water) group is shown in Table 4.

There are many antiepileptic drugs used currently but most of drugs are with drawbacks and limitations. The

Table 1: Effect of Flunarizine (Flu) on THLF in MES method

| | Duration of THLF (sec) | | Paired t-test | | |
|---|---|---|---|---|---|
| Groups | Before treatment | After treatment | t-values | p-values | Protection (%) |
| Parameters | Mean±SEM | Mean±SEM | | | |
| Distilled water (mg kg$^{-1}$) | 2.92±0.35 | 2.92±0.43 | 0.0230 | 0.9825 | 0 |
| FLU 5 | 4.00±1.05 | 2.52±0.37 | 1.622 | 0.1657 | 0 |
| FLU 10 | 2.58±0.18 | 1.57±0.51 | 2.173 | 0.0818 | 0 |
| FLU 15 | 3.35±0.55 | 1.10±0.36 | 2.866 | 0.0351 | 33.33 |

One way ANOVA: F = 0.9396, 3.827; p = 0.4401, 0.0257; *p <0.05

Table 2: Effect of Nimodipine (Nim) On THLF in MES method

| | Duration of THLF (sec) | | Paired t-test | | |
|---|---|---|---|---|---|
| Groups | Before treatment | After treatment | t-values | p-values | Protection (%) |
| Parameters | Mean±SEM | Mean±SEM | | | |
| 5% Tween 80 (mg kg$^{-1}$) | 4.02±0.57 | 4.19±0.48 | 0.6761 | 0.5290 | 0 |
| NIM 2.5 | 3.90±0.43 | 4.82±0.75 | 1.142 | 0.3051 | 0 |
| NIM 5 | 3.64±0.70 | 3.15±0.46 | 1.282 | 0.2560 | 0 |
| NIM 10 | 3.18±0.54 | 3.28±0.87 | 0.2611 | 0.8044 | 0 |

One way ANOVA: F = 0.4176, 1.395; p = 0.7423, 0.2733

Table 3: Effect of sodium valproate on stupor in MES method

| | Duration of stupor (sec) | | Paired t-test | | |
|---|---|---|---|---|---|
| Groups | Before treatment | After treatment | t-values | p-values | Protection (%) |
| Parameters | Mean±SEM | Mean±SEM | | | |
| Distilled water | 104.10±16.99 | 87.77±21.18 | 1.375 | 0.2276 | 0 |
| S.V 300 mg kg$^{-1}$ | 89.96±10.25 | - | 8.769 | 0.0003*** | 100 |

Unpaired t-test: p = 0.4926; t = 0.7123; ***p<0.001

Table 4: Effect of sodium valproate on latency period and duration of seizures in PTZ method

| | PTZ induced seizures (min.) | | |
|---|---|---|---|
| Groups | Latency period | Duration | Protection (%) |
| Parameters | Mean±SEM | Mean±SEM | |
| Distilled water | 1.5±0.2236 | 18.166±1.621 | 0 |
| S.V. 300 mg kg$^{-1}$ | 29±10.205 | 2±0.6325 | 33.33 |

Unpaired t-test: t-value = 2.694, 9.921; p = 0.0225*, ≤0.0001***; ***p<0.001, *p <0.05

most important limitations are their adverse effects, the therapeutic dose limits and compliance because of the need for long-term therapy. Calcium channel blockers have been used extensively in various cardiovascular disorders including ischemic heart disease, supraventricular arrhythmias, systemic hypertension, congestive heart failure and hypertrophic cardiomyopathy and also in non-cardiovascular conditions like migraine, nocturnal cramps, acute mountain sickness, vertigo, neuronal regenerative process, reversal of resistance in chemotherapy as well as chloroquine resistant malaria and as a tocolytic. In the present study, flunarizine was compared with its control (distilled water) and standard drug 300 mg kg$^{-1}$ sodium valproate in wistar rats by MES model. This reveals that flunarizine shows promising effect for decreasing duration of THLE. Though 15 mg kg$^{-1}$ flunarizine group has statistically similar effect as 300 mg kg$^{-1}$ sodium valproate group on duration of THLE but the efficacy seems to be lower. Dolin *et al*. (1988) mentions nitredipine and nimodipine significantly raised the threshold to pentylenetetrazol for up to 6 h after their injection and concluded that the calcium channel antagonists are anticonvulsant against only certain types of convulsions such as pentylenetetrazol.

## CONCLUSION

To conclude with results, our study revealed that cerebro selective calcium channel blockers 'Flunarizine' and 'Nimodipine' has promising anticonvulsant effect in both MES and PTZ Model which need to be explored. These drugs may be important for treatment of refractory epilepsy or when patients can't tolerate conventional antiepileptic drugs because of their adverse effects and the therapeutic drug monitoring required. CCBs can be used as add on therapy for treatment of different types of epilepsy with lower doses of conventional anti-epileptic drugs. More importantly they can be a potential add-on therapy to conventional antiepileptic drugs in treatment of refractory epilepsy.

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