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Research Article

ANTICONVULSANT ACTIVITY OF CALCIUM CHANNEL BLOCKERS ON EXPERIMENTALLY INDUCED SEIZURES IN ANIMAL MODELS

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ABSTRACT

Objectives: To evaluate the antiepileptic activity of calcium channel blockers in albino mice.

Methods: Swiss albino mice weighing around 25 g – 30 g of either sex were divided into 16 groups(n=6). They were divided into 2 equal parts for 2 methods each comprising 8 group. The anti-convulsant activity was screened using Maximal Electroshock Seizure (MES) model and Pentylenetetrazole (PTZ) model.

Results: Nifedipine (5 mg/kg body weight) when combined as an adjuvant with different doses of phenytoin (10 mg/kg body weight, 7.5 mg/kg body weight and 5 mg/kg body weight)/ Valproic acid (100 mg/kg body weight, 75 mg/kg body weight and 50 mg/kg body weight) has shown statistically significant ($P < 0.001$) anticonvulsant effect, both in Maximal Electro Shock (MES) method and Pentylene Tetrazole (PTZ) method.

In MES method, amlodipine (5 mg/kg body weight) is as potent as Nifedipine when used with different doses of Phenytoin (10 mg/kg body weight, 7.5 mg/kg body weight and 5 mg/kg body weight) in exhibiting statistically significant ($P < 0.001$) anticonvulsant effect only with one dose, i.e., 50 mg/kg body weight of valproic acid.

Conclusion: Nifedipine/Amlodipine when combined as an adjuvant with reduced dose of standard antiepileptic drugs has shown good results and this might help in minimizing the toxic effect of the standard antiepileptic drugs.

Keywords: Epilepsy; Valproic acid; Phenytoin, Maximal Electroshock Seizure model; Pentylenetetrazole.

INTRODUCTION

Epilepsy is one of the most common afflictions of man. With a prevalence of approximately 1%, it is estimated that 50 million people worldwide may have the disorder[1]. Even though it was recognized as early as 2000 B.C. new concepts about its pathogenesis, etiology and treatment are brought out almost every year[2].

In the past the treatment of epilepsy was based on superstition, religious beliefs and ignorance. But the present day concept of the treatment of convulsions is very much different from what it was earlier[3]. A large number of promising compounds are currently undergoing preclinical and clinical evaluation and several of these will undoubtedly become meaningful additions to the neurologists' pharmacological armamentarium[1].

Inspite of the vast number of drugs introduced for the treatment of epilepsy, there is still a need for an ideal antiepileptic agent with properties like broad spectrum activity, rapid onset of action, least side effects, good oral bioavailability and low cost[4]. Contemporary anticonvulsant therapy, however, is neither universally effective nor invariably safe. Their important adverse effects include central nervous system depression, ataxia, megaloblastic anaemia, cardiac arrhythmias, hepatic dysfunction and teratogenicity[5].

In the recent years, some investigators have reported that calcium channel blocker may prevent or suppress seizure induced by a variety of chemical or physical stimuli[6]. Physiological studies have emphasized the possible role of Ca++ flux on the paroxysmal discharges associated with seizure activity[7].

In neurons showing intrinsic burst firing, singalling epileptic activity there is a massive influx of Ca++ associated with the paroxysmal depolarizing shift (PDS) and hence the influx of extracellular Ca++ into neurons is considered to be an important feature in triggering epileptic activity[8,9]. Anticonvulsant such as phenytoin, barbiturates and benzodiazepines may act in part by inhibition of calcium influx and thus alter PDS[10]. Hence, Ca++ channel blockade may be important in preventing seizure spread. The above findings suggest that in refractory epilepsy, treatment with conventional antiepileptic drugs combined with agents which modify Ca++ modulation (viz., Ca++ antagonists), as add-on therapy, may provide better seizure control[11].

The dihydropyridine (DHP) class of calcium channel blocker antagonists provide a large group of structurally related compound with various levels of activity in which a structure activity relation can be determined[7].

In the light of the development cited above, an attempt has been made in this work to find out the effect of Nifedipine and Amlodipine (DHP class of calcium channel blockers) as an adjuvant along with the antiepileptic drugs in the prevention of experimentally induced seizures.

MATERIALS AND METHODS

Chemicals and solutions: Phenytoin, Valproic acid, Pentylene tetrazole, Nifedipine, Amlodipine, Propylene glycol, Distilled water

Animals: Albino mice of either sex, weighing between 20 – 30 gms were randomly selected from central animal facility, JSS Medical College, Mysore. Animals were housed in groups of 3 at an ambient temperature of 25± 1ºC with ad libitum access to food and water. The study protocol was approved by Institutional Animal Ethics Committee.

Equipments: Electro – convulsiometer with accessories, 1 ml syringes, Measuring jars and glass beakers, Electronic weighing balance**,** weighing balance, Animal cages.

Methodology

The anti epileptic activity of amlodipine and nifedipine were screened using maximal electroshock (MES) and Pentylenetetrazole (PTZ) Method.

Animals were divided into 16 groups, each group containing six animals. 16 groups were divided into 2 equal parts for 2 methods each comprising 8 group. These animals were subjected to maximal electroshock and chemoshock. All the preparations were administered intra-peritoneally. They were maintained under standard 12 hour light dark cycle. Experiments were carried out at around the same time each day.

Maximal electroshock induced seizures[12]

Procedure- Swiss albino mice weighing 25-30gm are used. Electrical stimulation is applied via ear electrodes with a current strength of 50 mA for 0.2 sec. The resultant seizure passes through various phases; phase of tonic limb flexion (1.5 sec duration), phase of tonic limb extension (10 sec duration), finally followed by variable short clonic interval which may lead to asphyxial death in some animals. 24 hours before testing of anticonvulsants (to avoid any possible kindling effect), the animals are pre-screened for their ability to develop full tonic extension in the maximal electroshock test. Only those animals showing hind limb tonic extension response will be used for maximal electroshock test. The same procedure will be followed for all the groups of mice.

Evaluation- Suppression of tonic hind limb extension is taken as measure of efficacy in this test.

C_1-Control group	0.25 ml. of Propylene glycol
S_1-Standard group	12.5 mg/kg of Phenytoin sodium
T_1-Test group	5 mg/kg of Nifedipine + 10 mg/kg of Phenytoin sodium
T_2-Test group	5 mg/kg of Nifedipine + 7.5 mg/kg of Phenytoin sodium
T_3-Test group	5 mg/kg of Nifedipine + 5 mg/kg of Phenytoin sodium
T_4-Test group	5 mg/kg of Amlodipine + 10 mg/kg of Phenytoin sodium
T_5-Test group	5 mg/kg of Amlodipine + 7.5 mg/kg of Phenytoin sodium
T_6-Test group	5 mg/kg of Amlodipine + 5 mg/kg of Phenytoin sodium

A pilot study was done to select the ED_{50} doses of the drugs (both standard as well as test drugs) used here.

Pentylenetetrazole (PTZ) Induced Convulsion

Procedure- Pentylenetetrazole is a central nervous system stimulant. The convulsive effect is analogous to petit mal type of convulsion in man. Albino mice of either sex of average weight 25 gms received an intraperitoneal injection of pentylenetetrazole (60 mg/kg body weight) [13] and the resulted seizures with its various phases to recovery were noted and timed.

The following parameters were recorded[14].

1) Seizure latency
2) Mild myoclonic jerks
3) Generalised clonic seizures with loss of righting reflex
4) Postictal depression followed by recovery/death.

Abolition of generalized clonic seizures with loss of righting reflex was taken as the index of protection[15,16].

C_2-Control group	0.25 ml of Propylene glycol
S_2-Standard group	110 mg/kg of Valproic acid
T_7-Test group	5 mg/kg of Nifedipine + 100 mg/kg of Valproic acid
T_8- Test group	5 mg/kg of Nifedipine + 75 mg/kg of Valproic acid
T_9- Test group	5 mg/kg of Nifedipine + 50 mg/kg of Valproic acid
T_{10}- Test group	5 mg/kg of Amlodipine + 100 mg/kg of Valproic acid
T_{11}- Test group	5 mg/kg of Amlodipine + 75 mg/kg of Valproic acid
T_{12}- Test group	5 mg/kg of Amlodipine + 50 mg/kg of Valproic acid

Statistical analysis

The effects of different drugs under study were analysed by calculating mean and S.D of the outcome parameters. One way Analysis of Variance (ANOVA) and independent samples T test is applied to see the difference between any two groups at a time.

Tests of significance were carried out at 5% level.

SPSS for windows (version 16) was applied in the statistical analysis.

RESULTS

Hind Limb Tonic Flexion

The mean time duration of hind limb tonic flexion was 1.5 sec. in control group (C_1). The mean time duration of hind limb tonic flexion was nil in standard and as well as test groups. i.e., (S_1, T_1, T_2, T_3, T_4, T_5, T_6). Hind limb tonic flexion was abolished in all these groups (Table I) and the percentage of protection conferred was 100% with S_1 , T_1 , T_2 , T_3 , T_4 , T_5 , T_6.

Hind Limb Tonic Extension

This parameter was observed in all animals of control (C_1) group and the mean time duration of this parameter was 12.83 seconds in control group (C_1).

Abolition of hind limb tonic extension was observed in all the animals of T_3 and T_6 (test) groups. The percentage of protection conferred by test drugs in T_3 and T_6 groups (Table II) was 100%.

Abolition of hind limb tonic extension was observed in all except one animal in the standard (S_1) group and test groups. (T_2 and T_5) and the mean time duration for S_1, T_2 and T_5 group was 83.3% (Table II).

In T_1 and T_4 groups, abolition of hind limb tonic extension was seen in all animals except two and the mean time duration was 3 seconds for both the groups. The protection conferred by the test drug in T_1 and T_4 group was 66.7% (Table II).

All the test groups and the standard groups have shown statistically significant protection ($P < 0.001$) against MES induced extensor phase as seen by the results (Table I).

Clonus

This parameter was observed in all the animals of control (C_1) and standard groups (S_1) and the mean time duration of this parameter in C_1 and S_1 was 10.5 seconds and 2.6 seconds respectively. But the mean time duration of S_1 group was very much decreased when compared to that of C_1 group and it is statistically significant ($P < 0.001$).

Among the test groups of animals, T_3 and T_5 groups exhibited profound reduction in the mean time duration of this phase i.e., 0.8 seconds and 1.3 seconds respectively and the percentage of protection conferred by test drug against MES induced convulsion in these groups was 83.3%

In T_2, T_4 and T_6 (test) groups, the mean time duration was 1.8 seconds, 3.3 seconds and 2.3 seconds respectively and the percentage of protection was 66.7%.

In T_1 group, though the mean time duration (i.e., 1.5 seconds) was reduced, 3 out of 6 animals exhibited this phase. All the test groups have shown statistically significant protection against MES induced clonic phase ($P < 0.001$) and ($P < 0.01$) (Table I).

Post Ictal Depression

This phase was observed in all the animals except one in control (C_1) group and the mean time duration was 94.6 seconds.

Among the test groups of animals, T_1, T_5 and T_6 groups exhibited profound reduction in the mean time duration of this phase (i.e 16.6 seconds, 18.3 seconds and 13.3 seconds respectively) and the percentage of protection conferred by test drugs against MES induced convulsions in these groups was 83.33% (i.e., one animal in each of these groups was unprotected). Test groups T_1, T_5 and T_6 have shown statistically significant protection ($P < 0.05$

and P < 0.01) against MES induced post ictal depression (Table I).

Whereas in standard (S_1) and the other test groups (T_2, T_3, T_4), this phase was abolished in all the animals and the percentage of protection conferred against MES induced convulsions was 100%.

Recovery/Death

The events such as hind limb tonic flexion, hind limb tonic extension, clonus and postictal depression were followed by recovery of all the animals and there was no death seen in any of the animals used in the MES method.

Table 1: Comparison of mean duration (in seconds) and standard deviation values of different parameters in MES method (Mean ± S.D.)

Parameters	Control	Standard	Test groups					
	C_1	S_1	T_1	T_2	T_3	T_4	T_5	T_6
Hind Limb Tonic Flexion	1.5 ± 0.55	--	--	--	--	--	--	--
Hind Limb Tonic Extension	12.83±1.47	1.83±4.491***	3±4.69***	0.83±2.04***	--	3±4.69***	1.33±3.27***	--
Clonus	10.5±1.05	2.67±0.82***	1.5±1.76***	1.83±2.86***	0.83±2.04***	3.33±5.16**	1.33±3.27***	2.33±3.67***
Post Ictal Depression	94.67±48.61	--	16.67±40.82*	--	--	--	18.33±44.91*	13.33±32.66*
Recovery/Death	R	R	R	R	R	R	R	R

n = 6, *P<0.05, **P<0.01, ***P<0.001

Stastical significance compared with control group; R = Recovery, D = Death

Table II: Percentage of protection conferred by the test drug in MES method

Serial No. of Groups	Control Group I	Standard Group II	Test Groups					
			Group III	Group IV	Group V	Group VI	Group VII	Group VIII
	C_1	S_1	T_1	T_2	T_3	T_4	T_5	T_6
Percentage of protection	0	83.33	66.7	83.33	100	66.7	83.33	100

Criterion: Abolition of hind limb tonic extension was considered to be protective end point.

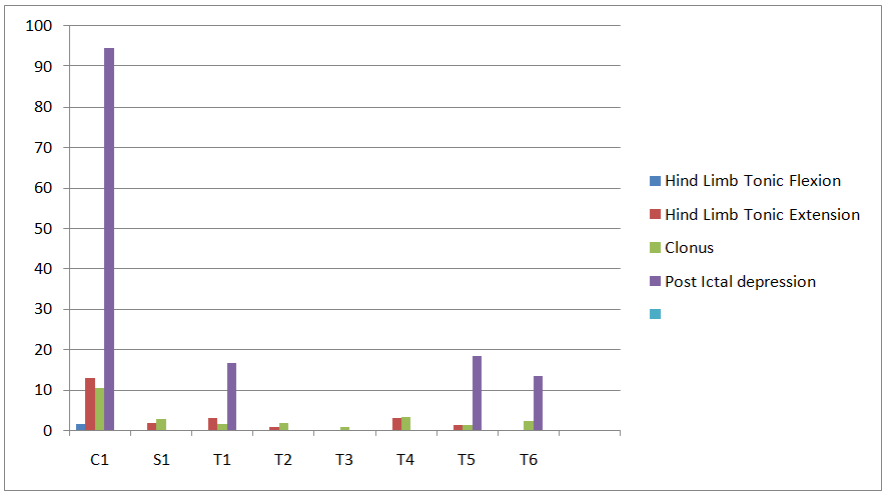

Fig. 1: Bar diagram showing Comparison of mean duration (in seconds) different parameters in MES method

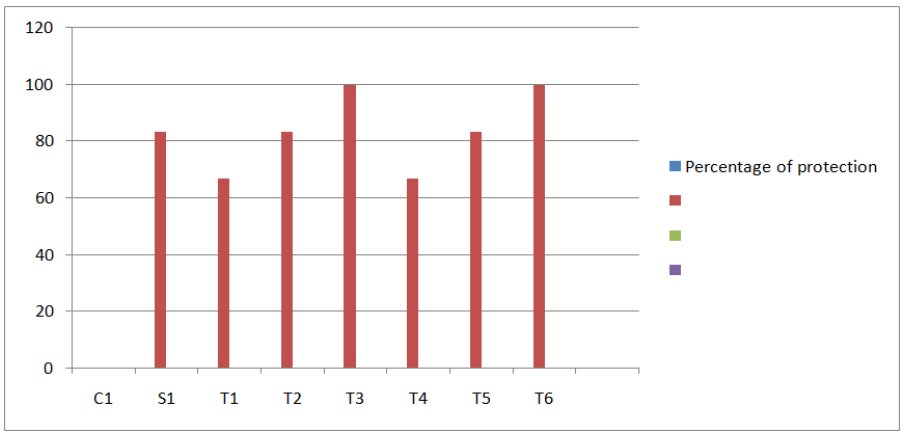

Fig. 2: Bar diagram showing Percentage of protection conferred by different groups in MES method

Table III: Comparison of mean duration (in seconds) and standard deviation values of different parameters in PTZ method (Mean ± S.D.)

Parameters	Control	Standard	Test groups					
	C_2	S_2	T_7	T_8	T_9	T_{10}	T_{11}	T_{12}
Seizure Latency	46.67±15.67	53±129.82	65±101.14	25±61.237	50±122.47	88.33±98.06	58.33±90.43	105±162.696
Mild Myoclonic Jerks	5.5±1.87	1±2.45**	5.33±8.64	1.67±4.08	1±2.45**	8.83±9.81	13.33±21.60	2±3.35
Generalised Clonic Seizures with loss of Righting Reflex	11.83±2.04	--	1.67±4.08***	1±2.45***	0.33±0.82***	5.33±5.89*	8.33±16.02	1.33±3.27***
Post Ictal Depression	185.33±15.88	29.33±71.85***	23.5±52.56***	13.33±32.66***	10±24.49***	36.67±57.15***	16.67±40.82	16.67±40.82***
Recovery/Death	**2D**	**R**	**R**	**R**	**R**	**1D**	**R**	**1D**

n = 6, *P<0.05, **P<0.01, ***P<0.001

Stastical significance compared with control groupR = Recovery, D = Death

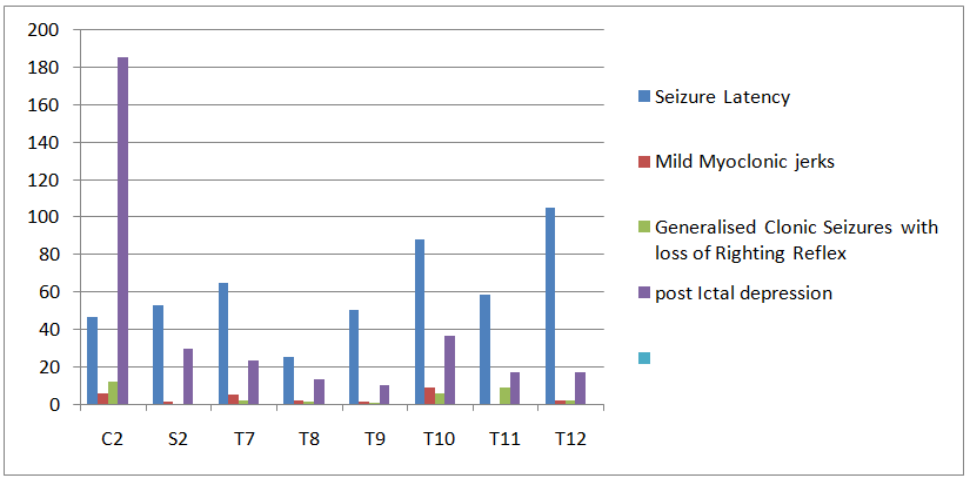

Fig. 3: bar diagram showing Comparison of mean duration (in seconds) different parameters in PTZ method

Table IV: Percentage of protection conferred by the test drug in PTZ method

Criterion: Prevention of generalized clonic seizures with loss of righting reflex was considered to be the protective end point.

Serial No. of Groups	Control Group IX	Standard Group X	Test Groups					
			Group XI	Group XII	Group XIII	Group XIV	Group XV	Group XVI
	C_2	S_2	T_7	T_8	T_9	T_{10}	T_{11}	T_{12}
Percentage of protection	0	83.33	83.33	83.33	83.33	50	66.7	83.33

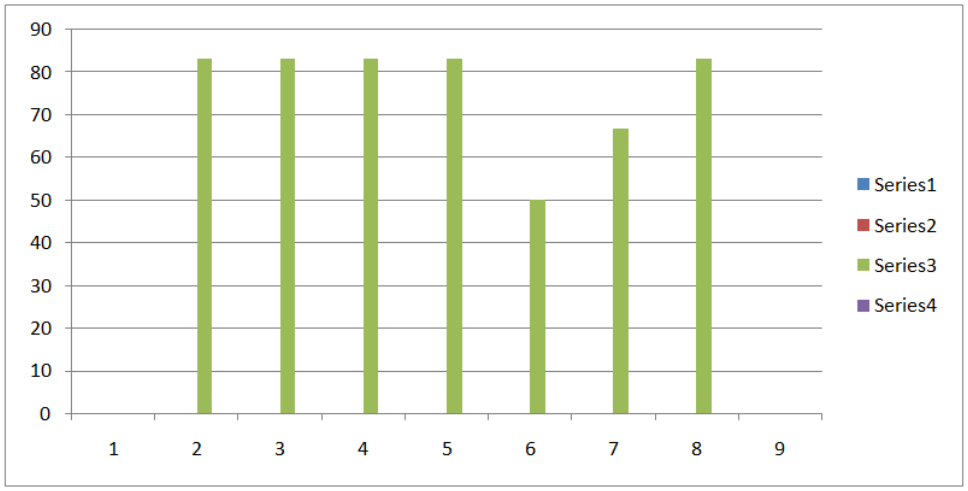

Fig. 4: Bar diagram showing Percentage of protection conferred by different groups in PTZ method

Seizure Latency

The mean time duration of seizure latency recorded in the control group (C_2) was 46.67 sec. in the standard group (S_2), all the animals except one were found to be protected (83.33%).

Among the (test) groups i.e., in T_8 and T_9, the mean time duration for this phase was 25 seconds and 50 seconds and in these groups all the animals except one in each group were found to be protected (83.33%).

In T_7, T_{11} and T_{12} (test) groups, the mean time duration was 65 seconds, 58.3 seconds and 105 seconds respectively but except two animals in each group, all the other animals were found to be protected (66.7%)

In T_{10} (test) group, the mean time duration was increased (88.3 seconds) and only three animals out of six were protected. Though the mean time duration in T_8 group was decreased when compared to control group the result was not statistically significant in this group and also in other groups including standard group (Table III).

Mild Myoclonic Jerks

The mean time duration of this phase in control group (C_2) group was 5.5 seconds. In the standard (S_2) group, the mean time duration (1 sec) and all the animals except one were protected against PTZ induced myoclonic jerks (83.33%) and was found to be statistically significant ($P < 0.01$) (TableI II).

Among the test groups i.e., in T_8 and T_9 the mean time duration was 1.6 seconds and 1 second respectively and the percentage of protection was 83.33% and the result in T_9 group was found to be statistically significant ($P < 0.01$) (Table II).

In T_7, T_{11} and T_{12} (test) groups, the mean time duration was 5.3 seconds, 13.3 seconds and 2 seconds and percentage of protection was 66.7% and the result in T_{12} group was statistically significant ($P < 0.05$) (Table II).

In T_{10} (Test) group, the mean time duration was increased (8.8 seconds) and only three animals were protected.

Generalized Clonic Seizures with loss of Righting Reflex

The mean time duration of this phase was 11.8 seconds in the control group (C_2). In the standard group (S_2), all animals were protected and the percentage of protection conferred by the standard group against PTZ induced generalized clonic seizures was 100%.

Among the test groups, the mean time duration of this phase was very much reduced in T_7, T_8, T_9, and T_{12} group (1.6 seconds, 1 second, 0.3 second and 1.3 seconds respectively). All the animals except one in each group were protected against PTZ induced seizures and the percentage of protection conferred by the test drug in different doses was 83.33% and these groups have shown statistically significant result ($P < 0.001$) (Table III).

In T_{11} (test) group, the mean time duration was 8.3 seconds and the percentage of protection was 66.7% in T_{10} group, the mean time duration was 5.3 seconds and has shown statistically significant protection ($P < 0.05$) against PTZ induced generalized clonic seizures (TableIII).

Postictal Depression

The mean time duration of this phase in the control (C_2) group was 185.33 seconds. In the standard (S_2) group, the mean time duration was reduced (29.3 seconds) and the percentage of protection was 83.33%.

In the test groups (T_7, T_8, T_9 and T_{12}), the mean time duration of this phase was 23.5 seconds, 13.3 seconds, 10 seconds and 16.6 seconds respectively. The percentage of protection conferred by all these group was 83.33%.

In T_{11} group, the mean time duration was 16.6 seconds and the percentage of protection was 66.7%. In T_{10} group, the mean time duration was 36.6 seconds and the percentage of protection was 50%.

All the test groups and the standard group have shown statistically significant protection ($P < 0.001$) against PTZ induced P.I.D (Table III).

Recovery/Death

Two deaths in the control (C_2) group and death of one animal each in T_{10} and T_{12} group were recorded. However the rest of the animals recovered in all the respective groups.

CONCLUSION

Nifedipine (5 mg/kg body weight) when combined as an adjuvant with different doses of phenytoin (10 mg/kg body weight, 7.5 mg/kg body weight and 5 mg/kg body weight)/ Valproic acid (100 mg/kg body weight, 75 mg/kg body weight and 50 mg/kg body weight) has shown statistically significant ($P < 0.001$) anticonvulsant effect, both in Maximal Electro Shock (MES) method and Pentylene Tetrazole (PTZ) method.

In MES method, amlodipine (5 mg/kg body weight) is as potent as Nifedipine when used with different doses of Phenytoin (10 mg/kg body weight, 7.5 mg/kg body weight and 5 mg/kg body weight) in exhibiting statistically significant ($P < 0.001$) anticonvulsant effect only with one dose, i.e., 50 mg/kg body weight of valproic acid.

Both Nifedipine and Amlodipine in the dose of 5 mg/kg body weight have conferred maximum protection rate i.e., 100% protection against MES method induced hind limb tonic extension phase when combined with 5 mg/kg body weight of Phenytoin Sodium.

Whereas in PTZ induced seizures, Nifedipine (5 mg/kg body weight) has conferred 83.33% protection when combined with different doses of valproic acid (100 mg/kg body weight, 75

mg/kg body weight and 50 mg/kg body weight). Amlodipine (5 mg/kg body weight) has conferred 83.33% protection against PTZ induced seizures when combined with only one dose of Valproic acid (i.e., 50 mg/kg body weight).

From this, it may be predicted that Nifedipine is probably useful in both grandmal epilepsy and petitmal epilepsy when used as an adjuvant. Whereas Amlodipine is more useful in grandmal epilepsy. And also the doses of the standard antiepileptic drugs can be reduced when they are concurrently used with Amlodipine/Nifidepine, so that the toxic effects of standard antiepileptic drugs can be minimized.

From the present study it may be speculated that Nifedipine/Amlodipine potentiates the antiepileptic activity of Phenytoin sodium and Valproic acid by blockade of voltage dependent calcium channles[7] and thus exerts a synergistic effect with a low dose of Phenytoin and Valproic acid.

However, studies with other models of epilepsy and in combination with various doses of conventional antiepileptic drugs on experimental animals and human beings would be needed with other groups of calcium channel blockers to substantiate the present work.

Nifedipine/Amlodipine when combined as an adjuvant with reduced dose of standard antiepileptic drugs have shown good results and this might help in minimizing the toxic effect of the standard antiepileptic drugs.

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