



Effects of Pregabalin, Nimodipine, and Their Combination in the Inhibition of Status Epilepticus and the Prevention of Death in Mice

Epilepsi Durumunun İnhibisyonuna ve Ölümün Önlenmesine Pregabalin, Nimodipin ve Bunların Kombinasyonunun Farelerdeki Etkileri

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ABSTRACT

Objectives: The current study aims to evaluate the combined antiepileptic effects of pregabalin (PGB) and nimodipine (NMD) in an acute seizure model of epilepsy in mice.

Materials and Methods: This study assessed the combined antiepileptic effects of PGB with NMD on death protection in mice. Pentylene-tetrazole was used to induce seizures. Both drugs were used singly and in combination to judge anticonvulsant effects on an acute seizure model of epilepsy in mice. Diazepam (DZ) and valproate (VPT) were used as standard antiepileptic drugs.

Results: The death protection in mice by both these drugs was observed in percentage and deliberated as marked change when the outcome of the tested drug was equal to ED₅₀ of PGB and measured highly marked when the result was more than ED₅₀ for PGB. Treatment with pregabalin and nimodipine combination revealed substantial mortality protection at 30+2.5 mg/kg dose and highly marked at doses from 35+5 mg/kg to 55+15 mg/kg, these effects were superior to individual effects of PGB, showing synergism, however lesser than classic drugs valproate and diazepam.

Conclusion: NMD showed synergistic anticonvulsant effect with PGB. However, clinical studies are required to establish the effectiveness of this combination in humans.

Key words: Pregabalin, nimodipine, valproate, diazepam, pentylene-tetrazole

ÖZ

Amaç: Bu çalışma, farelerde akut epilepsi nöbet modelinde pregabalin (PGB) ve nimodipinin (NMD) kombine antiepileptik etkilerini değerlendirmeyi amaçlamaktadır.

Gereç ve Yöntemler: Bu çalışma, farelerde ölümü önlemek için PGB ve NMD ile kombine antiepileptik etkilerini değerlendirmiştir. Bu çalışma, farelerde ölümü önlemek için PGB'nin NMD ile kombine antiepileptik etkilerini değerlendirmiştir. Pentilente-tetrazol nöbetleri indüklemek için kullanılmıştır. Farelerde epilepsinin akut nöbet modeli üzerindeki antikonvülsan etkileri değerlendirmek için her iki ilaç tek başına ve kombinasyon halinde kullanılmıştır. Standart antiepileptik ilaç olarak diazepam (DZ) ve valproat (VPT) kullanılmıştır.

Bulgular: Farelerde bu ilaçların her ikisi tarafından ölüm koruması yüzde olarak gözlemlendi ve test edilen ilacın sonucu PGB'nin ED₅₀'sine eşit olduğunda belirgin değişiklik olarak düşünüldü ve sonuç PGB için ED₅₀'den fazla olduğunda oldukça belirgin olarak ölçüldü. Pregabalin ve nimodipin kombinasyonu ile tedavi, 30+2,5 mg/kg dozda önemli mortalite koruması gösterdi ve 35+5 mg/kg ila 55+15 mg/kg dozlarda oldukça belirgindi, bu etkiler PGB'nin bireysel etkilerinden üstündü, ancak sinerjizm gösteriyordu. Klasik ilaçlar valproat ve diazepamdan daha az bulunmuştur.

Sonuç: NMD, PGB ile sinerjistik antikonvülsan etki göstermiştir. Bununla birlikte, bu kombinasyonun insanlarda etkinliğini belirlemek için klinik çalışmalara ihtiyaç vardır.

Anahtar kelimeler: Pregabalin, nimodipin, valproat, diazepam, pentilente-tetrazol

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Received: 01.05.2020, Accepted: 25.05.2020

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INTRODUCTION

Status epilepticus is a severe condition of shared epilepsies.¹ It is a life-threatening neural health crisis,² which may lead to loss of life in many instances if not cured suitably: Lorazepam and diazepam (DZ) are the major drugs of choice for a short period to control status epilepticus.³ However, lorazepam has been superior to DZ.⁴ Valproate (VPT), phenobarbitone, and phenytoin are endorsed for the extended period of treatment of status epilepticus.⁵ The selection of first-line drugs is limited to the management of status epilepticus, and the drugs suggested have serious side effects and drug-drug interactions.^{6,7} Nimodipine (NMD) is a voltage-gated calcium channel inhibitor permitted by Food and Drug Administration (FDA) for hypertension since it inhibits the L-type calcium channels in blood vessels.⁸

NMD inhibits and modifies voltage-gated calcium channels in the central nervous system (CNS).⁹ Low and high voltage-gated calcium channels have a crucial role in neuronal functions in major parts of the CNS, embracing excitability.^{10,11} Hence, it seems appropriate for the calcium-channel blockers (CCBs) to be employed to treat epilepsy.^{12,13} NMD in several animal studies showed increasing antiepileptic effects over other anti-convulsion drugs, e.g., carbamazepine, VPT, and lamotrigine, in the management of acute seizures.^{14,15} Pregabalin (PGB) is well tolerated, efficient and FDA permitted its use in patients with epilepsy.^{16,17} Its antiepileptic action relates to binding alpha-2-delta voltage-gated calcium channels, including many other pharmacodynamic anti-seizure actions in the CNS.¹⁸ The current study aims to evaluate the combined antiepileptic effects of PGB and NMD in an acute seizure model of epilepsy in mice.

MATERIALS AND METHODS

Animals

Consent was obtained from the Board of Advanced Studies and Research (BASR) 10 (P) 04 dated June 19, 2015, to conduct the current study at the Department of Pharmacology, University of Karachi. The mice used in the study were managed as per the guidelines of the National Institute of Health.¹⁹ The ethical approval for the use of animals was granted by the departmental research committee after the letter issued by the BASR dated September 30, 2015 (approval no: BASR/02521). The experiments were performed on male albino mice whose weight ranged from 20 to 25 g. The animals were kept in a controlled environment with regular provisions of food and water. The temperature was sustained at 21°C±2°C with 12-hour light/dark cycles. The animals were exposed to the location for 2-3 days before the experiments could be performed. The tests were done from 9:00 am to 12:30 pm.

Drug treatment

Acute attacks were induced by pentylenetetrazole (PTZ). Meanwhile, PGB and NMD were used as test drugs. DZ and VPT were employed to equate the antiepileptic effects of the test drugs. Normal saline was used for animals in the control

group. The HEJ Research Center, University of Karachi, provided PTZ. DZ and VPT injections were acquired from a local market by Roche Pakistan Ltd. and Abbot Laboratories, Pakistan Ltd, Karachi, respectively. PGB capsules of Getz Pharma Pvt. Limited, Karachi were also obtained from the local market. Novartis Pharma, Pakistan Ltd, Karachi, provided the NMD injections.

PGB, NMD, and PTZ were liquefied in sterile, normal saline. Aluminum foil was used to cover the PTZ solution to avoid disintegration. The solutions were made regularly and used within an hour of their inception. PGB capsule contents were dissolved in sterile water and administer subcutaneously, while other drugs were given by the intraperitoneal route.

Procedure

Convulsions in all mice were induced via PTZ at a dose of 90 mg/kg through subcutaneous administration.²⁰ PGB and NMD were given separately and in combination as six different doses. All drugs, including the test substance and the standard drugs DZ and VPT, were administered 40 minutes earlier than PTZ, administered intraperitoneally. The mice were kept alone following PTZ administration and observed. The prevention of death was recorded in percentage.

The outcomes of mice that remained without seizures up to cut-off time were stated as 0. The number of mice survived (mortality protection) and died were recorded as a percentage. The anticonvulsant effect, seizure form, and mortality protection achieved by the combination regimen (PGB + NMD + PTZ) in six doses of the test drugs were equated with PTZ, individual effects of PGB + PTZ and NMD + PTZ, and with reference drugs (DZ + PTZ and VPT + PTZ).

A period of 60 minutes was the cut-off time for seizure defense after PTZ administration with or without the test drugs.²¹⁻²⁵ The antiepileptic effects of PGB and NMD were assessed by observing seizure prevention as a percentage.

All animals used in this study were distributed into three parts, i.e., A, B, and C, each part had ten groups, and each group included 12 mice. Group I in each part functioned as control and was given sterile, normal saline; group II mice were given PTZ, and animals in groups III to VIII were given different doses of the test drugs. Mice in groups IX and X were given DZ and VPT. DZ was given at a dose of 7.5 mg/kg and VPT 100 mg/kg, forty minutes before giving PTZ. Six groups of part-A were given PGB doses ranging from 30 to 55 mg/kg, with a difference of 5 mg/kg in each group. Animals of part-B were given NMD at doses of 2.5, 5, 7.5, 10, 12.5, and 15 mg/kg. Animals in part-C were given six different combinations of PGB + NMD, i.e., 30+2.5, 35+5, 40+7.5, 45+10, 50+12.5, and 55+15 mg/kg, forty minutes before giving PTZ.

Statistical analysis

No standard statistical procedure was performed in the current study. The results were expressed in percentages and produced a noticeable outcome when the tested drugs were equal to effective dose 50 (ED₅₀) of PGB and a highly substantial result when the outcome was more than ED50 for PGB.

RESULTS

Table 1 summarizes the results of all animals groups in the three sections however Figure 1 shows the results of animals only in section-A. PGB as a single agent in its six doses from 30 to 55 mg/kg protected mortality from 42% to 67%. Both the reference drugs VPT and DZ provided 100% mortality protection. The antiepileptic effects of PGB, when matched to the reference drugs VPT and DZ, were comparatively inferior in preventing all epileptic attacks.

Table 1 summarizes the results of all animals groups in the three sections however Figure 2 shows the results of animals only in section-B. NMD as a single agent in its first three doses, i.e., 2.5, 5, and 7.5 mg/kg, exhibited 100% mortality. However, at a dose of 10 mg/kg, the mortality protection was 8%, whereas at doses of 12.5 mg/kg and 15 mg/kg, the mortality protection was 17%. VPT and DZ prevented death in 100% of animals. The antiepileptic effect of NMD, when matched to standard drugs VPT and DZ, was highly inferior in all seizure forms.

Table 1. Antiepileptic outcome of pregabalin, nimodipine, and their combination in an acute model of epilepsy

Groups	Doses mg/kg	Mortality protection
Normal control	10 mL/kg	100
PTZ	90	00
PGB	30	42
PGB	35	42
PGB	40	50*
PGB	45	58**
PGB	50	67**
PGB	55	67**
NMD	2.5	00
NMD	5.0	00
NMD	7.5	00
NMD	10	8
NMD	12.5	17
NMD	15	17
PGB:NMD	30:2.5	50*
PGB:NMD	35:5	58**
PGB:NMD	40:7.5	58**
PGB:NMD	45:10	67**
PGB:NMD	50:12.5	83**
PGB:NMD	55:15	83**
VPT	100	100
DZ	7.5	100

*Marked as compared to 40 mg/kg PGB (ED₅₀), **Highly substantial as compared to 40 mg/kg PGB (ED₅₀), n=12, PTZ: Pentylene tetrazole, PGB: Pregabalin, NMD: Nimodipine, VPT: Valproate, DZ: Diazepam, ED₅₀: Effective dose 50

Table 1 summarizes the outcomes of all animals groups in the three sections. In contrast, Figure 3 shows the results of animals in section-C. The animals treated in combination dose of 30+2.5 mg/kg PGB and NMD prevented death in 50%, which was substantial compared with PGB 40 mg/kg. In contrast, animals treated with combination doses of 35+5 and 40+7.5 mg/kg PGB and NMD, respectively, showed a 58% mortality protection. The animals given the combination of PGB and NMD at doses of 45+10 mg/kg revealed a 67% mortality protection. However, the maximum mortality protection of 83% was observed by the combination doses of 50+12.5 mg/kg and 55+15 mg/kg of doses of PGB and NMD, respectively.

Table 2 and Figure 4 show the difference in the mortality protection among PGB, NMD, the combination of PGB + NMD,

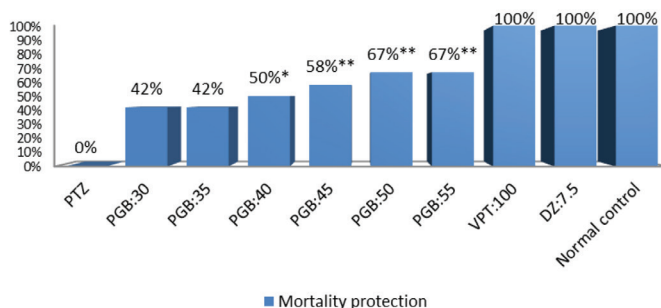


Figure 1. Comparative effects of pregabalin, valproate, and diazepam on mortality protection in an acute model of epilepsy

*Shows marked change and **shows highly substantial change in terms of percentage, PTZ: Pentylene tetrazole, PGB: Pregabalin, VPT: Valproate, DZ: Diazepam

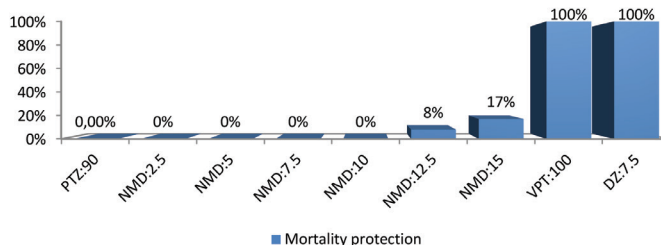


Figure 2. Comparative effects of nimodipine, valproate, and diazepam on the mortality protection in an acute model of epilepsy

PTZ: Pentylene tetrazole, NMD: Nimodipine, VPT: Valproate, DZ: Diazepam

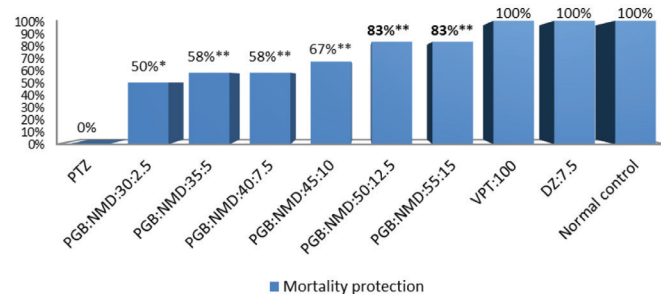


Figure 3. Comparative effects of pregabalin, nimodipine, valproate, and diazepam on the mortality protection in an acute model of epilepsy

*Marked as compared to 40 mg/kg PGB (ED₅₀), **Highly substantial as compared to 40 mg/kg PGB (ED₅₀), PTZ: Pentylene tetrazole, NMD: Nimodipine, VPT: Valproate, DZ: Diazepam, ED₅₀: Effective dose 50

and VPT. The difference in the mortality protection among NMD in the first three doses and VPT was -100. In contrast, NMD demonstrated a 92% less mortality protection compared to VPT at 10 mg/kg and 83% less mortality protection at 12.5 and 15 mg/kg. PGB demonstrated a 58% less mortality protection at 30 and 35 mg/kg doses reduced to 50% at the dose of 40 mg/kg. While at the dose of 45 mg/kg, a 42% less mortality protection was noted. However, at 50 and 55 mg/kg doses, PGB demonstrated 33% less mortality protection than VPT. The combination of PGB and NMD showed better results in reducing mortality. The difference in mortality protection at doses of 30+2.5 mg/kg and 35+5 mg/kg compared with VPT was only 50%, which improved to 42% at the combination dose of 40+7.5 mg/kg. The difference in the mortality protection improved to 33% at doses of 45+10 mg/kg and was reduced to 17% at the combination doses of 50+12.5 mg/kg and 55+15 mg/kg compared with VPT.

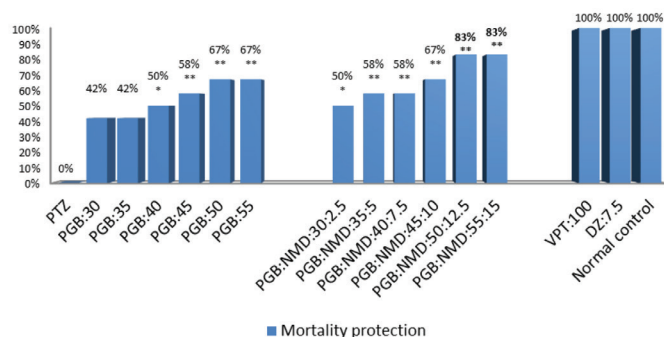


Figure 4. Comparative effects of pregabalin, nimodipine, valproate, and diazepam on the mortality protection in an acute model of epilepsy

*Marked as compared with 40 mg/kg PGB (ED₅₀), **Highly substantial compared with 40 mg/kg PGB (ED₅₀), PTZ: Pentylentetrazole, PGB: Pregabalin, NMD: Nimodipine, VPT: Valproate, DZ: Diazepam, ED₅₀: Effective dose 50

Table 3 shows the comparisons of the prevention of death for PGB and combinations of PGB and NMD. The PGB and NMD combination at the dose of 30+2.5 mg/kg demonstrated an 8% higher prevention of death, whereas the combination dose of 35+5 mg/kg showed a 16% higher prevention of death. However, the combination doses of 40+7.5 and 45+10 for PGB and NMD demonstrated only 8% and 9% prevention of death, respectively. The rate of prevention of death was again consistently higher. It was up to 16% higher with the doses combination of 50+12 mg/kg and 55+15 mg/kg of PGB and NMD compared with PGB alone.

DISCUSSION

Although the discovery of new antiepileptic drugs (AEDs) have widened the selection of drugs to treat epilepsies on a long-term basis, none of the newer AEDs are permitted to treat status epilepticus. Also, the likelihood and usefulness of these new AEDs remain limited, and no AED has been found to retain its best properties.²⁶ Exploration of drug combinations that may have the potential to terminate and prevent status epilepticus is the main object of our study. Thus, newer AEDs with better effectiveness and unique mechanisms are now needed to deliver active combinations to treat epilepsy.²⁷ Animal models may only be employed to forecast drugs combinations that are effective in the clinical setting.²⁴

AEDs may have inhibitory or additive effects. However, drugs possessing additive effects appear to be of medical importance.^{21,28} Therefore, the present investigation evaluated PGB in combination with NMD for synergistic antiepileptic effects in an acute seizure model of epilepsy in mice. It was assumed that a combination of AEDs might potentiate their anticonvulsant effects. This hypothesis was supported by the additive actions of tiagabine and gabapentin. The outcomes

Table 2. Nimodipine, pregabalin, and PGB + NMD in comparison to valproate in the mortality protection in an acute model of epilepsy

NMD v/s VPT dose mg/kg	Difference in mortality protection	PGB v/s VPT	Difference in mortality protection	PGB:NMD v/s VPT dose mg/kg	Difference in mortality protection
NMD v/s VPT 2.5 100	-100	PGB v/s VPT 30:100	58	PGB:NMD v/s VPT 30:2.5/100	50
NMD v/s VPT 5 100	-100	PGB v/s VPT 35:100	58	PGB:NMD v/s VPT 35:5/100	42
NMD v/s VPT 7.5 100	-100	PGB v/s VPT 40:100	50	PGB:NMD v/s VPT 40:7.5/100	42
NMD v/s VPT 10 100	92	PGB v/s VPT 45:100	42	PGB:NMD v/s VPT 45:10/100	33
NMD v/s VPT 12.5 100	83	PGB v/s VPT 50:100	33	PGB:NMD v/s VPT 50:12.5/100	17
NMD v/s VPT 15 100	83	PGB v/s VPT 55:100	33	PGB:NMD v/s VPT 55:15/100	17

Difference in the mortality protection (%), PGB: Pregabalin, NMD: Nimodipine, VPT: Valproate

Table 3. Comparison of the mortality protection pregabalin and PGB + NMD in an acute model of epilepsy

Treatment groups	Mortality protection	Treatment groups with doses	Mortality protection	Difference in the mortality protection
PGB:30	42	PGB:NMD 30:2.5	50*	8
PGB:35	42	PGB:NMD 35:5	58**	16
PGB:40	50	PGB:NMD 40 :7.5	58**	8
PGB:45	58	PGB:NMD 45:10	67**	9
PGB:50	67	PGB:NMD 50:12.5	83**	16
PGB:55	67	PGB:NMD 55:15	83**	16

*Marked compared with 40 mg/kg PGB (ED₅₀), **Highly substantial compared with 40 mg/kg PGB (ED₅₀), n=12, doses (mg/kg), PGB: Pregabalin, NMD: Nimodipine, ED₅₀: Effective dose 50

of the present investigation demonstrated that the activation of the neurotransmitters might have produced an additive effect.²⁹ In another investigation, the anticonvulsant effects of oxcarbazepine and gabapentin were found to be a mixture of both drugs at the fixed ratios to exert the additive effects against electroconvulsions.³⁰

PGB and NMD showed a 50% prevention of death when given a combination dose of 30+2.5 mg/kg. However, when PGB and NMD were given separately at doses of 30 mg/kg and 2.5 mg/kg, the prevention of death was 42% and 0%, respectively. Hence, there was an 8% increase in the prevention of death compared with PGB and a 50% increase compared with NMD alone (Table 1). Similarly, PGB and NMD showed a 58% prevention of death at the combination dose of 35+5 mg/kg. However, when these drugs were given separately at doses of 35 mg/kg and 5 mg/kg, the prevention of death was 42% and 0%, respectively. Therefore, a 16% increase in the prevention of death compared with PGB and a 58% increase in the response compared with NMD in PTZ-induced acute seizures (Table 1).

PGB and NMD showed a 67% prevention of death with the combination dose of 45+10 mg/kg. However, when PGB and NMD were given separately at doses of 45 mg/kg and 10 mg/kg, the prevention of death was 58% and 8%, respectively. Hence, there was a 9% increase in the prevention of death compared with PGB and a 59% increase in the prevention of death compared with NMD (Table 1). The animal groups received combination doses of 50+12.5 mg/kg and 55+15 mg/kg PGB. NMD exhibited maximum anticonvulsant effects with an 83% prevention of death. This was 17% and 67% more than the individual response to PGB and NMD, respectively. Hence, synergistic effects have been observed at all the combination doses of PGB and NMD. Thus, the combination regimen decreased the ED₅₀ of the PGB from 40 mg/kg to 30 mg/kg. The decrease in ED₅₀ was 10 mg/kg, which was a 25% decrease in the ED₅₀ of the PGB in the treatment of acute seizures in mice (Table 1). This shows that

adding the CCB NMD with PGB exerted remarkable synergistic anti-seizure effects by increasing the mortality protection but not abolishing the seizures completely.

The pharmacokinetic and pharmacodynamic profiles of PGB reveal it to be a well-tolerated drug.^{17,31,32} The FDA has recognized it for the treatment of epilepsy.^{33,34} PGB in several investigations has validated its value for the treatment of partial and generalized seizures. PGB has also shown its effectiveness in refractory epilepsy, and combination treatment may have a broader range to manage many forms of convulsions.¹⁶ In this study, we anticipated that the antiepileptic actions of PGB could be amplified or altered when administered in combination with NMD. Calcium channel antagonists retain anticonvulsant effects in experimental models and enhance the guarding activity of some AEDs.³⁵ NMD is a dihydropyridine calcium channel antagonist that blocks N- and P/Q-type calcium channels, with greater affinity for both channels.^{36,37} Numerous investigations have shown important augmented anticonvulsant actions of amlodipine and NMD on topiramate, VPT, phenobarbitone, and other AEDs.^{15,30,38} Thus, there are convincing reasons illustrating that this investigation provides an essential medical perspective.

PGB as monotherapy in acute studies showed marginal to moderate effects on the prevention of death. The effects were inferior compared with the efficacy of VPT and DZ at all test doses. No doses of PGB as monotherapy in acute studies demonstrated a 100% prevention of death against PTZ. Moreover, the prevention of death with PGB monotherapy in the present study was dose-dependent. PGB demonstrated minimum prevention of death of 42% at 30 and 35 mg/kg, which was 58% less than both reference drugs. The prevention of death was enhanced to 50% at 40 mg/kg, 50% less than VPT and DZ. The maximum prevention of death of 67% by PGB was observed at the doses of 50 and 55 mg/kg in acute seizures but was 33% less than both reference drugs. The efficacy of PGB in acute seizures was 42% to 67% compared with the efficacy of VPT (Table 1, Figure 4).

Low-voltage-gated calcium channels have a wide distribution throughout the CNS and contribute to major processes like neuronal excitability and synaptic transmission.³⁹ Among the dihydropyridines, NMD blocks both the L- and T-type calcium channels.⁴⁰ The present study results suggest that the CCB NMD had some involvement in reducing seizures and the death rate, probably by inhibiting T-type low-voltage calcium channels. Therefore, the combination of PGB and NMD revealed greater antiepileptic activity compared with their individual effects at the same doses.

CONCLUSION

The antiepileptic combination used in the current study demonstrated marked seizure protection overall. However, PGB and NMD at the combination dose of 50+12.5 mg/kg showed maximum prevention of death, which was 83%. Therefore, it may be concluded that the CCB NMD exhibited anticonvulsant effects by potentiating the antiepileptic effects of PGB in the combination. The primary clinical importance of combination therapies would be a reduction in PGB doses with several times increase in efficacy.

The present study results provide clear evidence that the combination of PGB and NMD provides a better option to treat status epilepticus and typical and atypical epilepsies. The combination of PGB and NMD may have substantial potential to treat diverse types of epilepsies comprising resistant epilepsies due to channel-modifying effects. The major advantage of combination therapy would be the low doses of PGB with better anti-seizure effects. The essential point of this study is the use of combination regimens of PGB/NMD for the short- and long-term control of status epilepticus. However, this may require further animal and clinical studies.

Conflict of interest: No conflict of interest was declared by the authors. The authors are solely responsible for the content and writing of this paper.

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