

## Evaluation of anticonvulsant effects of nimodipine and ascorbic acid on pentylenetetrazole- induced seizures in mice

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### ABSTRACT

**Introduction:** The mechanisms underlying the vulnerability of the brain to seizures remains unknown. Calcium ions influx and oxidative stress have been implicated in a variety of acute and chronic epileptogenic conditions. The present study was aimed at investigating the effects of nimodipine and ascorbic acid (vitamin C) alone and in combination on pentylenetetrazole (PTZ) - induced seizures in mice.

**Material and methods:** The animals received nimodipine (0.5, 1, 1.5, 2 mg/Kg, i.p.), ascorbic acid (30, 100, 300 mg/Kg, i.p.) alone and in combination with sodium valproate (100 mg/Kg, i.p.), 15 and 30min prior to intra-peritoneal injection of PTZ (60 mg/Kg) and the acute seizure parameters such as seizure latency, duration and protection percent were studied in each group.

**Results:** Ascorbic acid alone did not have any effects on the seizure parameters and the number of mice convulsing (  $P>0.05$ ). Nimodipine in 2 mg/kg dose had full protective effect on PTZ- induced seizure parameters, and in lesser doses it exerted partial protective effects. The combination of ascorbic acid (300 mg/Kg) with nimodipine (1.5 mg/Kg) or sodium valproate had a significant synergistic protective effect against PTZ- induce seizures in comparison with controls(  $P<0.001$ ).

**Conclusion:** Ascorbic acid potentiates the anticonvulsive effects of nimodipine on PTZ-induced seizure in mice.

**Keywords:** Nimodipine, Ascorbic acid, Pentylenetetrazole, Seizures, Mice

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## INTRODUCTION

Epilepsy is one of the most frequent chronic diseases of central nervous system with a prevalence of approximately 1% of the human population (1). Seizures have been known as occasional discharges in the nervous tissue, characterized by recurrent paroxysmal changes in the neurological action caused by abnormalities in the electrical activity of the brain (1). The main mechanism of induction and propagation of epileptic episodes is unclear, but there are many hypotheses on seizure pathophysiology. Excessive calcium influx has been implicated in the pathophysiology of epilepsy and there are considerable evidences that calcium is an important factor for induction of epilepsy (2-4). Previous studies have shown that the blockers of voltage dependent Calcium channels such as dihydropyridine calcium channel blockers (CCBs) display anticonvulsant activity in various models of experimental convulsions (5-8) and in humans (9).

Another recently proposed mechanism of seizures is the generation of free radicals and oxidative stress during seizures. The role of reactive oxygen and nitrogen species in the generation and spread of convulsions is not established, but many studies have suggested that the oxygen reactive species may play important role in the genesis and /or maintenance of seizures (10-13).

Drug therapy is the most important strategy in the treatment of this disorder, but many of antiepileptic drugs have serious side effects (1); therefore, research on new anticonvulsive drugs is continuing. From this point of view, co-administration of a calcium channel blocker and an antioxidant agent could be effective in the control of seizures and might have synergistic effect in combination therapy. For this propose, the present study was designed to determine combined effects of nimodipine and ascorbic acid (vitamin C) on pentylenetetrazole (PTZ)-induced seizures in mice.

## MATERIAL AND METHODS

### *Animals:*

Experiments were carried out on adult male mice weighing between 20-30 g, obtained from Pasture Institute of Iran. The animals were kept in polystyrene cages under standard laboratory conditions (temperature:  $22 \pm 2^\circ\text{C}$  and 12 hr photoperiod). They had free access to food and water, except during the test period. Each experimental group consisted of 10 mice.

### *Drugs:*

Nimodipine (0.5, 1, 1.5, and 2 mg/kg, i.p., Bayer Health Care Co., Germany), sodium valproate (VPA) (100mg/kg, Sigma Chemical Co. USA), PTZ (60 mg/kg, i.p., Sigma Chemical Co., USA) and ascorbic acid (30, 100 and 300 mg/kg, i.p., Osve Pharmaceutical Co., Tehran, Iran).

Ascorbic acid, sodium valproate and PTZ were dissolved in normal saline. Nimodipine was dissolved in Tween 80 and diluted with distilled water.

### *PTZ induced seizures in mice:*

The mice were administered PTZ (60 mg/kg, i.p.) and they were observed for 30 minutes for the tonic-clonic seizures. The seizure latency (SL), seizure duration (SD), protection percent and Hind Limb Extension (HLE) were evaluated as seizure parameters described by Khanna (8). The pre-treatment of animals with ascorbic acid, nimodipine, saline, and VPA (as standard anticonvulsive drug) and their combination was performed 15 and 30 minutes prior to each PTZ treatment in separate groups. Reduction in convulsive movements and the seizure parameters were selected as the criteria for evaluation of anticonvulsive activity of the drugs.

### *Statistical analysis:*

Data were represented as mean  $\pm$  SE for 10 mice in each group. Data were analyzed using one-way analysis of variance (ANOVA) (Tukey's test) using SPSS version 13. P values less than 0.05 were considered statistically significant.

**RESULTS**

PTZ (60 mg/ kg) in the control group which received normal saline 15 and 30 min prior to PTZ, produced tonic- clonic convulsions in 100% of animals with seizure latencies of  $137.5 \pm 19.8$  and  $214.9 \pm 24.3$  seconds which lasted for nearly  $12.1 \pm 0.7$  and  $12.4 \pm 1.4$  seconds, respectively (Tables 1 and 2, Fig. 1 and 2).

PTZ produced tonic-clonic convulsions in 80% and 71% of animals pretreated with sodium valproate as standard anticonvulsive drug 15 and 30 minutes before PTZ injection, respectively (Fig. 1 and 2).

Vitamin C did not show any anticonvulsive effects when it was administered alone in all dose categories (Fig 1 and 2, Tables 1 and 2).

**Table 1: Effects of nimodipine and ascorbic acid (vitamin C) on pentylenetetrazole(PTZ) induced seizures in mice (administered 15 min before PTZ injection)**

Groups	Seizure Latency (Sec)	Seizure Duration (Sec)	No. of animals with H.L.E
Normal saline (1 ml/100g)	$137.5 \pm 19.8$	$12.1 \pm 0.7$	0
Sodium valproate (100 mg/kg)	$263.7 \pm 27.4$	$10.5 \pm 2.0$	2
Nimodipine (0.5 mg/kg)	$337.2 \pm 98.3$	$14.9 \pm 1.9$	0
Nimodipine (1 mg/kg)	a **** $535 \pm 98.2$	$10.4 \pm 2.5$	0
Nimodipine (1.5 mg/kg)	a *** b**** $695.8 \pm 122$	$8.6 \pm 3.0$	0
Nimodipine (2 mg/kg)	Protected		
Vitamin C (30 mg/kg)	$171.3 \pm 13.3$	$13.3 \pm 1.0$	0
Vitamin C (100 mg/kg)	$181.3 \pm 13.7$	$13.3 \pm 0.8$	0
Vitamin C (300 mg/kg)	$162.2 \pm 20.7$	$12.9 \pm 1.8$	0
Nimodipine (1.5 mg/kg) +Vitamin C (300 mg/kg)	Protected		
Nimodipine (1.5 mg/kg) + Vitamin C (300 mg/kg) + Sodium Valproate (100 mg/kg)	a*** b** $716.7 \pm 141$	a** b* $2.4 \pm 1.3$	0

Values have shown as Mean $\pm$  SEM

\* $p < 0.05$ , \*\* $p < 0.01$  and \*\*\* $p < 0.001$  when compared to the control group A and b represent significant differences with normal saline and sodium valproate, respectively (N=10).

H.L.E= Hind Limb Extension

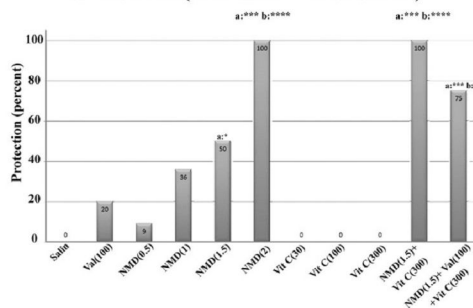
**Table 2:** Effects of nimodipine and ascorbic acid (vitamin C) on pentylenetetrazole (PTZ) induced seizures in mice (administered 15 min before PTZ injection)

Groups	Seizure Latency (Sec)	Seizure Duration (Sec)	No. of animals with H.L.E
Normal saline (1 ml/100g)	214.9 ± 24.3	12.4 ± 1.4	0
Sodium valproate (100 mg/kg)	270.6 ± 41	7.5 ± 2.3	0
Nimodipine (0.5 mg/kg)	196.8 ± 35.2	11.3 ± 1.5	1
Nimodipine (1 mg/kg)	248.7 ± 46.3	11.4 ± 2.4	1
Nimodipine (1.5 mg/kg)	a*** b** 507.3 ± 113.5	7.3 ± 2.3	0
Nimodipine (2 mg/kg)	Protected		
Vitamin C (30 mg/kg)	130.7 ± 13.7	14.9 ± 1.1	0
Vitamin C (100 mg/kg)	130.9 ± 10.4	12.9 ± 1.1	0
Vitamin C (300 mg/kg)	180.5 ± 16.6	10.4 ± 0.4 a**	0
Vitamin C (300 mg/kg) + Sodium Valproate (100 mg/kg)	418.7 ± 4.3	2.4 ± 1.2	0
Nimodipine (1.5 mg/kg) + Vitamin C (300 mg/kg)	Protected		
Nimodipine (1.5 mg/kg) + Vitamin C (300 mg/kg) + Sodium Valproate (100 mg/kg)	Protected		

Values have shown as Mean ± SEM

\* $p < 0.05$ , \*\* $p < 0.01$  and \*\*\* $p < 0.001$  when compared to the control groups. A and b represent significant differences with normal saline and sodium valproate, respectively (N=10). H.L.E= Hind Limb Extension

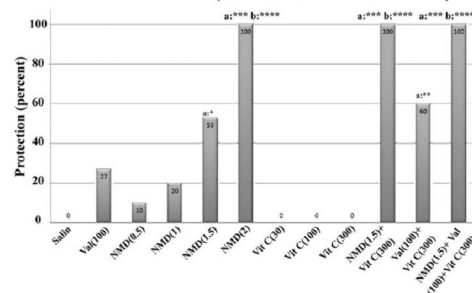
**Fig 1:** Protective effects of nimodipine and ascorbic acid (vitamin C) against pentylenetetrazole (PTZ) induced seizures in mice (administered 15 min before PTZ)



\* $p < 0.05$ , \*\* $p < 0.01$  and \*\*\* $p < 0.001$  when compared to the control group A and b represent significant differences with normal saline and sodium valproate, respectively (N=10). Values in the parentheses represent the dose of the drugs (mg/kg).

Salin=Saline, Val=Sodium valproate, NMD=Nimodipine, Vit C=Vitamin C

**Fig 2:** Protective effects of nimodipine and ascorbic acid (vitamin C) against pentylenetetrazole (PTZ) induced seizures in mice (administered 30 min before PTZ)



\* $p < 0.05$ , \*\* $p < 0.01$  and \*\*\* $p < 0.001$  when compared to the control group A and b represent significant differences with normal saline and sodium valproate, respectively (N=10). Values in the parentheses represent the dose of the drugs (mg/kg).

Salin=Normal saline, Val= Sodium valproate, NMD= Nimodipine, Vit C= Vitamin C

A significant increase in seizure latency and decrease in seizure duration parameters were observed by nimodipine pretreatment alone with 1, 1.5 and 2 mg/kg doses in comparison with controls ( $p < 0.01$ ) and in comparison with normal saline and sodium valproate groups respectively ( $p < 0.001$ ) (Fig. and Tables 1 and 2). Nimodipine produced full anticonvulsive effects on PTZ-induced seizure when administered in 2 mg/kg dose ( $p < 0.001$ ) (Fig. and Tables 1 and 2).

Combination therapy in animals with nimodipine (1.5 mg/kg) and vitamin C (300 mg/kg) demonstrated a significant synergistic effect against PTZ-induced seizures ( $p < 0.001$ ) (Fig. and Tables 1 and 2).

When high dose nimodipine and vitamin C (1.5 and 300 mg/kg, respectively) were administered in combination with standard antiepileptic drug (sodium valproate 100 mg/kg), further significant reduction in the seizure parameters was seen as compared to the control animals and those treated with either nimodipine or vitamin C alone ( $p < 0.001$ ) (Fig. and Table 2).

## DISCUSSION

The results of the present study indicate that nimodipine had anticonvulsant activity but vitamin C was not effective when injected alone on the PTZ-induced seizures model but its combination with nimodipine produced synergistic and marked protective effects on PTZ-induced acute seizures.

Our findings about anticonvulsive effects of nimodipine on PTZ-induced acute seizures are similar to previous studies (7, 14-17). Nimodipine is a centrally active calcium antagonist that blocks the voltage-dependent L-type channels. Its antiepileptic properties have been proved in various animal models. For example, nimodipine attenuates excitability by blocking calcium influx through voltage-dependent L-channels secondary to kainic acid-induced membrane depolarization (14) and the combination of nimodipine and antiepileptic drugs may provide more efficient protection against

PTZ-induced seizures in mice (16). In another study, it was demonstrated that nimodipine inhibited pilocarpine-induced convulsions and status epilepticus, and significantly decreased the percentage of deaths and cerebral changes (17). These studies suggest that the modulation of the calcium influx in CNS by nimodipine might influence the epileptic phenomena. The electrophysiological evidence has indicated that calcium ion currents in neuronal cells are sensitive to voltage dependent calcium channel antagonists (16, 17). Moreover, depending on the experimental convulsion model, the central effects of voltage dependent CCBs show some anticonvulsant activity. (18)

Recent studies have revealed the important role of oxidative stress and free radicals overproduction during seizures in experimental models (10-13, 19). Previous experiments have shown that the generation of free hydroxyl radicals in rat brain homogenates is increased following pentylenetetrazol (PTZ) kindling and reactive oxygen species (ROS) have recently been suggested to play a critical role (20). Free radicals, as well as nitric oxide synthase (NOS), are implicated in PTZ-induced kindling and antioxidants could be effective in controlling the accompanying changes (21). The effects of PTZ administration on the thiol redox state, lipid peroxidation, and protein oxidation in the mouse striatum have been established by previous studies (19). In this study we also evaluated the oxidative stress and a significant decrease in thiol redox state (decrease in glutathione, glutathione disulfide, cysteine, and protein thiols) and increased lipid peroxidation occurred after PTZ-induced seizure. Therefore, administration of antioxidants may be potentially beneficial for the treatment of convulsive states. Many researchers have investigated the potential anticonvulsive effects of some antioxidant agents (19). One study indicated that alpha-tocopherol in a dose-dependent manner can attenuate striatal content of thiobarbituric acid-reactive substances (TBARS) and

total protein carbonylation in PTZ and methylmalonate-induced convulsion models in rats (20).

Ascorbate is an antioxidant vitamin that is found in high concentrations in the brain and seems to have neuroprotective properties in some experimental models of excitotoxic neurological disorders, including convulsive behavior and reactive species-related damages (22). Ascorbate, at high doses (300 mg/kg), protects against PTZ-induced convulsions, protein carbonylation, and inhibition of  $\text{Na}^+, \text{K}^+$ -ATPase activity in the rat striatum. Conversely, intermediate doses of ascorbate (100 mg/kg) potentiates the duration of convulsive episodes but has no additive effects on protein carbonylation or  $\text{Na}^+, \text{K}^+$ -ATPase activity inhibition induced by PTZ. Low doses of ascorbate (30 mg/kg) prevents PTZ-induced increase in total striatal carbonyl protein content, but does not alter PTZ-induced convulsions and  $\text{Na}^+, \text{K}^+$ -ATPase activity inhibition. These data indicates that the anticonvulsant activity of ascorbate is not related to its antioxidant action and supports a dual role for this compound as a neuroprotective agent, since while it protects against PTZ-induced cellular oxidative damage, it has a biphasic effect on PTZ-induced convulsions (22). In contrast in our study, vitamin C when used alone did not have any protective effect against acute PTZ-induced seizures in the above mentioned doses and it might be related to animal species differences and drug administration time courses (22).

In our study co-administration of moderate dose of nimodipine with high dose of vitamin C synergistically protected the animals from PTZ-induced acute seizures. Previous studies have demonstrated that nimodipine has antioxidant effects in experimental epilepsy models but it cannot reduce lipid peroxidation and striatal catalase activity in rats via blockade of calcium channels after pilocarpine-induced seizures (23). Therefore, we believe that vitamin C potentiates anticonvulsive effects of nimodipine through its

antioxidant effects, although further investigations for determining oxidative stress status in these experimental models is necessary.

## CONCLUSION

The present study indicated that anticonvulsant activity of nimodipine can be augmented with vitamin C co-administration. These drugs may have important potentials as adjuvant, nonsedative antiepileptic drugs, especially in those patients that conventional therapy has been inadequate. Further experimental and clinical studies to substantiate these findings and establish side effects and optimal doses are needed.

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