IJPSR (2020), Volume 11, Issue 4 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 03 June 2019; received in revised form, 03 November 2019; accepted, 06 February 2020; published 01 April 2020

COMBINED ANTICONVULSANT EFFECT OF NIFEDIPINE AND PENTAZOCINE IN EXPERIMENTALLY INDUCED CONVULSIONS BY ELECTRO CONVULSOMETER IN MICE AND RATS

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Keywords:

Anticonvulsant, Maximal electroshock, Electro convulsometer, Seizures, Nifedipine, Pentazocine

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ABSTRACT: The present study aimed to investigate the combined anticonvulsant effect of nifedipine (calcium channel blocker) and pentazocine (opioid analgesic) on the duration of convulsions, tonic hind limb extension and recovery in mice and rats. The study was initiated after obtaining ethical approval from the Institutional Animal Ethics Committee (IAEC), Department of Pharmacology, Osmania Medical College, Koti, Hyderabad. The anticonvulsant effect of these drugs were screened using Maximal Electro-Shock (MES) method and animals showing tonic hind limb extension response were divided into groups (six animals per group) in both species. Both mice and rats (6 animals/group) were treated with nifedipine (10 mg/kg), pentazocine (30 mg/kg), a combination of nifedipine (10 mg/kg) and pentazocine (30 mg/kg) and control animals are given distilled water as vehicle. The drug administered by an intraperitoneal route. The data were analyzed using ANOVA and group means were compared with LSD Post Hoc Test. P values < 0.05 were considered as significant. The animals treated with both nifedipine 10 mg/kg and pentazocine 30 mg/kg showed a significant reduction in the duration of convulsions and tonic hind limb extension (THLE) in mice and rats as compared to other groups. The results obtained in this study provide supporting pharmacological evidence of efficacy, the possible potential benefit of combining nifedipine with pentazocine in epilepsy.

INTRODUCTION: Epilepsy is a serious neurological condition characterized by recurrent unprovoked seizures that affect approximately 60 million people worldwide [1].

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DOI: 10.13040/IJPSR.0975-8232.11(4).1845-49

The article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.11(4).1845-49

The prevalence of epilepsy is 0.7% in India. The WHO has estimated that approximately 80% of the people with epilepsy live in developing countries, and most of them do not get adequate medical treatment [2].

Despite many advances in epilepsy research, the pharmacotherapy of epilepsy remains largely empirical, owing to the lack of understanding of the underlying pathology. These limitations with the conventional antiepileptic drugs (CAED) alone highlighted the need for exploring the drugs that

could potentiate the action of CAEDs to make the treatment of epilepsy more effective [3].

Calcium channel blockers (CCBs) may have a crucial role in the treatment of epilepsy. The initiation of epileptogenic activity in the neuron involves the phenomenon known as "intrinsic burst firing" activated by the inward Ca^{2+} current. Ca^{2+} is the primary mediator of excitotoxic neuronal damage during the seizure activity.

A fall in the extracellular calcium concentrations occurs before the onset of seizure activity followed by an increase in the intracellular calcium concentrations [4]. Hence, the 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 that modify Ca^{++} modulation (*viz.,* Ca^{++} antagonists), as add-on therapy, may provide better seizure control [5]. It is interesting to note that mu, delta and kappa opioids selectively hyperpolarize neurons through-out the CNS. Therefore, it seems possible that opioid peptides may indeed form as an endogenous anticonvulsant in the CNS modulating the underlying mechanisms of seizures arrest and refractoriness which are critical to the suppression of convulsions [6]. Pentazocine is known to act as an agonist at kappa-opioid receptors and a weak antagonist or a partial agonist at Mu receptors. This has shown significant anticonvulsant activity in maximal electroshock test [7].

Most of the convulsions can be controlled with available anticonvulsant drugs, few of them still remain resistant to treatment. Recognizing this, there is a need to develop newer antiepileptic drugs with therapeutic potential. The Present experiment was to evaluate the anticonvulsant effect of nifedipinea calcium channel blocker, pentazocine an opioid analgesic, and combined effect of nifedipine and pentazocine in experimentally induced seizures by Maximal Electro-Shock (MES) in mice and rats.

MATERIALS AND METHODS:

Drugs and Solutions: Nifedipine (Depin) 5 mg capsule manufactured by Zydus Cadila Healthcare Limited and Pentazocine (For twin) 1 ml ampoule manufactured by Ranbaxy Laboratories were used for anticonvulsant activity. Normal Saline (0.9% NaCl solution). Nifedipine 5 mg was dissolved in Tween 80 and diluted to 1 ml with double distilled water.

Laboratory Animals: Swiss albino mice (20-40 g) and Wistar rats (150-200 g) were procured from National Center for Laboratory Animal Sciences (NCLAS), ICMR-National Institute of Nutrition, Hyderabad after obtaining approval from Institutional Animal Ethics Committee (IAEC No: omc/pharma/IAEC/2007/05), Department of Pharmacology, Osmania Medical College, Koti, Hyderabad. The environmental conditions were maintained at 22 ± 2 °C, with relative humidity 50 – 55% with a 12 h light/dark cycle. The animals were fed on sterile pellet feed and purified water *ad libitum* both being withdrawn just prior to experimentation.

Electrically Induced Seizure in Mice and Rats: MES stimulation was done by an electroshock apparatus (Electro Convulsometer by Techno Electronics) through trans-corneal electrodes at an intensity sufficient to produce tonic hind limb extension (THLE). The current variable from 0.25 to 360 MA, +10% is suitable for producing minimal and supramaximal seizures required in the assay of anticonvulsant and anti-epileptic drugs [6].

In both mice and rats, seizures were induced with an alternating current of 50 mA and 150 mA respective intensity for 0.2 sec through corneal electrodes (before using a drop of normal saline was applied to corneal electrodes). Various phases of seizures *i.e.,* onset and type to recovery, were noted and timed and the parameters like duration of convulsions, duration of THLE and postictal depression followed by recovery.

Experimental Design: Only those animals showing THLE responses were considered for the experiment. Both mice and rats (6 animals/group) were treated with nifedipine (10 mg/kg body weight), pentazocine (30 mg/kg body weight), a combination of nifedipine (10 mg/kg) and pentazocine (30 mg/kg) and control animals are given distilled water as vehicle. Each animal received 0.2 ml volume by intraperitoneal route. After 45 min of drug administration, the animals were subjected to MES with an alternating current

of an intensity of 50 mA (mice) and 150 mA (rats) for 0.2 seconds through corneal electrodes and observed for the positive response. The positive response was considered when the tonic hind limb extension response was abolished or decreased.

Statistical Analysis: The results are expressed as mean ± SD. The data were analyzed using SPSS (Statistical Package for the Social Sciences) 15.0 windows version using a one-way analysis of variance (ANOVA) followed by group means comparison with LSD Post Hoc Test. P values < 0.05 were considered as statistically significant.

RESULTS: In the present study, the combined effect of nifedipine (10 mg/kg) and pentazocine (30 mg/kg) has shown a significant reduction in the mean duration of convulsions, tonic hind limb extension and recovery as compared to the control, nifedipine and pentazocine **Table 1-3** and **Fig. 1-2** in both mice and rats.

A combination of nifedipine and pentazocine has a synergistic effect. Thus, it may be effective against MES induced convulsions in mice and rats.

TABLE 1: COMBINED EFFECT OF NIFEDIPINE AND PENTAZOCINE ONMES INDUCED CONVULSIONS IN MICE

Treatment	Duration of Convulsions	Duration of THLE	Duration of Recovery
Control	42.17 ± 2.563	16.33 ± 1.862	7. 00 ± 1.414
Nifedipine	40.33 ± 1.751	13.17 ± 1.169*	5.66 ± 0.816*
Pentazocine	39.83 ± 1.835	13.50 ± 1.871*	6.17 ± 0.753
Nifedipine+ Pentazocine	34.66 ± 3.141**	10.83 ± 2.317**	4.33 ± 1.033**

The data are expressed as Mean ± SD; Six animals in a group (n=6); **statistically significant (P<0.01) compared to Control

TABLE 2: COMBINED EFFECT OF NIFEDIPINE AND PENTAZOCINE AGAINST MES INDUCED CONVULSIONS IN RATS

Treatment	Duration of Convulsions	Duration of THLE	Duration of Recovery
Control	41.67 ± 1.751	16.17 ± 1.472	7.50 ± 1.049
Nifedipine	38.50 ± 1.871*	14.33 ± 1.862	6.33 ± 1.033
Pentazocine	39.33 ± 1.033*	14.17 ± 1.472*	6.17 ± 1.169
Nifedipine+ Pentazocine	36.33 ± 1.751**	10.33 ± 1.633**	5.17 ± 1.169**

The data are expressed as Mean ± SD; Six animals in a group (n = 6); **statistically significant (P < 0.01) compared to Control; *statistically significant (P < 0.01) compared to Control

TABLE 3: MULTIPLE COMPARISON BY POST HOC LSD TEST SHOWING SIGNIFICANT DIFFERENCE BETWEEN THE GROUPS

Variable	Groups		Mice P- value	Rats P- value
Duration of Convulsion	Control	Nifedipine	0.199	<0.003*
		Pentazocine	0.106	<0.023*
		Nifedipine + Pentazocine	<0.000**	<0.000**
	Nifedipine	Pentazocine	0.721	0.388
		Nifedipine + Pentazocine	< 0.001 **	<0.033*
	Pentazocine	Nifedipine + Pentazocine	<0.001**	<0.005*
Duration of THLE	Control	Nifedipine	<0.008*	0.064
		Pentazocine	<0.015*	<0.045*
		Nifedipine + Pentazocine	<0.000**	<0.000**
	Nifedipine	Pentazocine	0.758	0.860
		Nifedipine + Pentazocine	<0.041*	<0.000**
	Pentazocine	Nifedipine + Pentazocine	<0.021*	< 0.001**
Duration of Recovery	Control	Nifedipine	<0.038*	0.083
		Pentazocine	0.179	0.050
		Nifedipine + Pentazocine	<0.000**	<0.002**
	Nifedipine	Pentazocine	0.413	0.797
		Nifedipine + Pentazocine	<0.038*	0.083
	Pentazocine	Nifedipine + Pentazocine	<0.006**	0.133

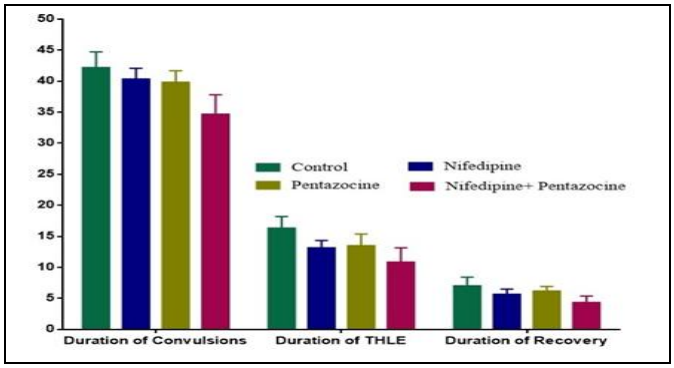

FIG. 1: COMBINED EFFECT OF NIFEDIPINE AND PENTAZOCINE ON MES INDUCED CONVULSIONS IN MICE

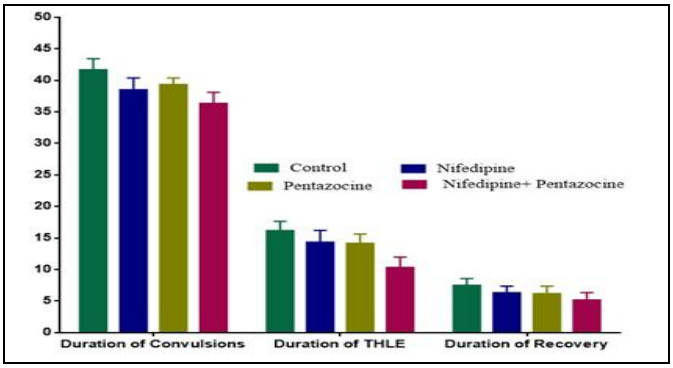

FIG. 2: COMBINED EFFECT OF NIFEDIPINE AND PENTAZOCINE ON MES INDUCED CONVULSIONS IN RATS

DISCUSSION: The experiment has been planned with an objective to study the combined anticonvulsant effect of Nifedipine and Pentazocine in experimentally induced seizures by MES stimulation through trans-corneal electrodes at an intensity sufficient to produce tonic hind limb extension (THLE) in mice and rats. The mice and rats are treated with nifedipine at a dose of 10 mg/kg and pentazocine at a dose of 30 mg/kg by intraperitoneal injection. After 45 min the animals were subjected to MES to induce seizures. Various phases of seizures like onset and type to recovery were noted and timed and the parameters like duration of convulsions, duration of THLE and postictal depression followed by recovery were recorded. The decrease or abolition in the duration of THLE was taken as the Index of anticonvulsant activity.

The data analyzed using one-way analysis of variance (ANOVA) followed by multiple comparison with LSD Post Hoc Test showed that mean duration of convulsions and duration of THLE were significantly (P-value < 0.001) reduced in nifedine + pentazocine group compared to control group whereas a significant reduction of $p<0.05$ compared to nifedipine and pentazocine groups in both the species. The mean duration of recovery showed significant ($p<0.001$) reduction in nifedine + pentazocine group compared to the control group whereas a significant reduction of $p<0.05$ compared to nifedipine and pentazocine groups in mice and $p<0.05$ compared to nifedipine group in rats. Many of the currently used antiepileptic drugs have been shown to block the calcium channels and the present study also demonstrated that Nifedipine has anticonvulsant action, calcium channels are more commonly viewed as attractive targets for novel epileptic therapies [8]. In view of promising results with pentazocine, the screening and evaluation of highly selective kappa-opioid drugs may prove beneficial to the development of novel therapeutic approaches to epilepsy [9, 10]. The results of the present study demonstrate that combined effect of nifedipine a calcium channel blocker and pentazocine, an opioid analgesic has elicited a significant reduction in the mean duration of convulsions, duration of THLE and duration of recovery against control, nifedipine and pentazocine in both mice and rats. Based on the above results, it can be suggested that nifedipine in combination with pentazocine may be effective against partial and generalized tonic-clonic seizures [11, 12].

CONCLUSION: Many of the previous studies suggested that nifedipine (calcium channel blocker) and pentazocine (opioid analgesic) has got anticonvulsant activity, the current study was conducted to see the combined effect of nifedipine and pentazocine in experimentally induced seizures by MES stimulation compare to nifedipine, pentazocine and control which received distilled water. The results obtained in this study shows that combined effect of nifedipine (10 mg/kg/bw) and pentazocine (30 mg/kg/bw) has decreased mean duration of convulsions, tonic hind limb extension and recovery time when compared with nifedipine and pentazocine alone in both mice and rats. The outcome of this study provides supporting pharmacological evidence of efficacy, a possible potential benefit of combining nifedipine with pentazocine in epilepsy. However, further studies are required to encourage the use of a combination of nifedipine and pentazocine in seizures.

ACKNOWLEDGEMENT: Authors like to thank Dr. B. Kavitha Devi (Assistant Professor, Department of Pathology, Osmania Medical College, Hyderabad) and Ms. T. Lalitha (Technical Assistant, PCT, ICMR-NIN, Hyderabad) for their contribution and support in the study.

CONFLICTS OF INTEREST: No conflict of interest.

REFERENCES:

1. Damasceno DD, Ferreira AJ, Doretto MC and Almeida AP: Anti-convulsant and anti-arrhythmic effects of Nifedipine in rats prone to audio genic seizures. Braz J Med Biol Res 2012; 45(11): 1060-65.
2. Carl W and Timothy AP: Advances in the medical treatment of epilepsy. Annu Rev Med 1988; 49: 135-62.
3. Ranjana IB, Vikrant VW, Swanand SP and Kartik JS: Role of Cinnarizine and Nifedipine on anticonvulsant effect of Sodium Valproate and Carbamazepine in maximal electroshock and pentylenetetrazole model of seizures in mice. J Pharmacol Pharmacother 2010; 1(2): 78-81.
4. Roopa B, Janardhan M and Karunasree P: Effect of calcium channel blocker as anticonvulsant and its potentiating effect when used along with sodium valproate in Pentylenetetrazole induced seizures in Albino rats. IJBCP 2018; 7(4): 714-17.
5. Kalabharathi HL, Sushma VN, Pushpa VH and Satish AM: Anti-convulsant activity of calcium channel blockers on experimentally Induced seizures in animal models. Int J Pharm Pharm Sci 2014; 6(1): 541-46.
6. Maddala R, Balu J and Viswashanthi A: Study of antiepileptic effect of pentazocine and combination of pentazocine with phenytoin in experimentally induced convulsions in albino rats. IOSR-JDMS 2017; 16(6): 51-54.
7. Praveen P, Shailender S and Siddappa D: Study on the anticonvulsant activity of pentazocine in albino rats. J Chem Pharm Res 2011; 3(5): 468-72.
8. Raina J, Ashish J and Prashant W: Anti-convulsant effects of nifedipine on MES induced seizures. Int J Curr Pharm Res 2018; 10(3): 37-38.
9. Anshu M, Sharma KK and Mediratta PK: The anticonvulsant effect of Pentazocine against maximal electroshock seizure in mice. IJP 1997; 29: 194-97.
10. Church J and Fletcher EJ: Blockade by sigma site ligands of high voltage-activated Ca^{2+} channels in rat and mouse cultured hippocampal pyramidal neurones. British journal of pharmacology 1995; 116(7): 2801-10.
11. Zamponi GW, Striessnig J, Koschak A and Dolphin AC: The physiology, pathology and pharmacology of voltage-gated calcium channels and their future therapeutic potential. Pharmacological reviews 2015; 67(4): 821-70.
12. MuralidharKhanna N, Khosla R and Kohli J: Opioid receptor mediated anticonvulsant effect of pentazocine. Indian J Med Sci 1998; 52(1): 1-7.

How to cite this article:
Kumar SB, Rajanna A and Balakrishna N: Combined anticonvulsant effect of nifedipine and pentazocine in experimentally induced convulsions by electro convulsometer in mice and rats. Int J Pharm Sci & Res 2020; 11(4): 1845-49. doi: 10.13040/IJPSR.0975-8232. 11(4).1845-49.