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Evaluation of analgesic activity of t type calcium channel blocker (ethosuximide) in animal model of neuropathic pain

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Abstract
Background: Neuropathic Pain is recognized as one of the most difficult type of pain to treat with conventional analgesics. Several evidence implicate that T-type VGCC in Pathophysiology of neuropathic and inflammatory pain. Ethosuximide, a T type VGCC blocker thus can be useful in Neuropathic Pain.
Objectives
1. To evaluate the analgesic activity of Ethosuximide in Normal rats.
2. To evaluate the analgesic activity of Ethosuximide in Vincristine induced animal model of neuropathic Rats.

Material & Methods
Drugs: Ethosuximide, Vincristine.
Animals: Adult albino rats (wt.180-200gms, of either sex) were divided in 5 groups of ten rats in each group.
Group 1 was Normal rats: Group 2 was vehicle treated group; Group 3 was Vincristine induced neuropathy; Group 4 was given Different doses of Ethosuximide intraperitoneally on different days in Normal rats; Group 5 was neuropathy-induced rats who were given different doses of Ethosuximide i.p on different days.
Evaluation of analgesic activity of Ethosuximide was carried out by using acetone drop method (ADM), cold Tail immersion test (TIT), hot plate method (HPM) in Normal and neuropathy induced rats.
Results: Ethosuximide (95 mg/kg, 125 mg/kg, 155 mg/kg, 190 mg/kg &220 mg/kg, i.p) was effective in alleviating Thermal algesia in Normal rats (HPM, TIT).There was no Allodynia. Cold Allodynia and thermal Hyperalgesia were exhibited after ten days of Vincristine administration (50mcg/kg, daily, i.p, 10days). Intraperitoneal administration of Ethosuximide (95 mg/kg, 125 mg/kg, 155 mg/kg, 190 mg/kg &220 mg/kg) attenuated the thermal Hyperalgesia (TIT, HPM) in dose dependent manner in Neuropathic rats. It also alleviated cold Allodynia (ADM) in Neuropathic rats.
Conclusion: Ethosuximide has analgesic activity in acute pain model. It is also effective in relieving thermal Hyperalgesia and cold Allodynia in Vincristine induced neuropathic pain in rats.

Keywords: Neuropathic pain, ethosuximide, vincristine

1. Introduction
Pain is the most common symptom reported in both the general population and the general medical setting [1-3]. Neuropathic Pain is a chronic pain syndrome caused by drug, disease or injury induced damage or destruction of sensory neurons with the dorsal root ganglia of the peripheral nervous system. One characteristic of neuropathic pain is its resistance to common analgesics and is currently the focus of intense research aimed at developing alleviating treatments [4].
A large body of data has clearly indicated that Voltage gated Calcium Channel (VGCC) are implicated in mediating various disease states [5, 6], including pain processing [7, 8-11] Ethosuximide, a selective VGCC blocker thus holds a promise as analgesic agent in Acute and chronic (neuropathic) Pain.

2. AIMS and Objectives
1. To evaluate the analgesic activity of Ethosuximide in Normal rats

2. To evaluate the analgesic activity of Ethosuximide in Vincristine induced animal model of neuropathic Rats.

3. Materials & Methods

This study was conducted after approval of Institutional animal Ethics Committee of Sri Aurbindo Medical College, Indore.

Animals: Healthy adult Albino rats weighing 180-250 gm of either sex were used.

Drugs: Ethosuximide &Vincristine (sigma labs), double distilled water (DDW)

Methods

(a) Induction of Neuropathic pain by Vincristine:

Administration of Vincristine (50µg/kg i.p. OD) in rats for 10 consecutive days, which lead to neuropathy [12].

(b) Behavioral Examinations

i) Paw cold Allodynia (acetone drop method [ADM])

The cold Allodynia was assessed in different groups by spraying a 100µL of acetone onto the planter surface of the paw, without touching the skin. The duration of response will be recorded with an arbitrary minimum value of 0.5s and a maximum of 20s [12].

ii) Paw Heat Hyperalgesia (Hot Plate Test [HPM])

The thermal nociceptive threshold, as an index of thermal Hyperalgesia (algesia in normal rats) was assessed by the Eddy's Hot Plate. The Plate was heated and maintained at temperature of 52.5±2.0 ºC. The rats were placed on Hot Plate and nociception, with respect to licking of hind paw, was recorded. The cut off time was 20 seconds.[13]

iii) Tail Cold Hyperalgesia test (Tail immersion test [TIT])

The Tail Cold Hyperalgesia (algesia in normal rats) was noted by immersing a terminal part of the rat tail (1 cms) in the water maintained at temperature of 0.4 ºC (mixed with ethylene glycol 1:1).The tail withdrawal latency was recorded and a cut off time was 20 seconds [14].

Experimental Protocol

8 Groups, each group comprising 10 albino rats were used in this study.

Group 1. Normal Control group.

Rats were not subjected to any treatment. The behavioral test were performed on different days i.e. days 2, 6, 8 &10.

Group 2. Distilled Water control group (DDW).

One ml *i.p*. of Double distilled water was administrated to normal rats for 10 days. The behavioral test were performed on different days i.e. days 2, 6, 8 & 10 Group 3. Different doses of Ethosuximide in Normal Rats.

Ethosuximide (95 mg/kg, 125 mg/kg, 155 mg/kg, 190 mg/kg &220 mg/kg, i.p) was administered to Normal rats. Each dose was administered on days 2, 6, 8, & 10 and behavioral test were performed.

Group 4. Vincristine treated group (Neuropathy group).

Vincristine (50µg/kg *i.p*.) was administrated to 50 normal rats for 10 days to induce neuropathy. To record baseline values the behavioral tests were performed on 10 rats after induction of neuropathy on different day's i.e. days 2, 6, 8, &10.

Group 5. Different doses of Ethosuximide in Vincristine treated group (Neuropathy group)

Ethosuximide (95 mg/kg, 125 mg/kg, 155 mg/kg, 190 mg/kg &220 mg/kg, i.p.) was administered to Vincristine treated group (Neuropathy group). Each dose was administered on days 2, 6, 8, & 10 and behavioral test were performed.

Statistical Analysis

The Means ±S.E.M of Different groups was compared to find 'p' values using two-tailed student's unpaired 't' test.

P values <0.05 were considered significant, p values <0.01 were considered very significant and p values <0.001 were considered to be extremely significant.

4. Results

Table 1: Comparison of Different Doses of Ethosuximide in Normal rats

	Day second		Day sixth		Day eight		Day tenth	
	HPM	**TIT**	**HPM**	**TIT**	**HPM**	**TIT**	**HPM**	**TIT**
Normal	9.085± 1.836	8.326± 2.034	11.378 ± 1.782	5.77± 1.879	8.711± 1.617	6.454± 1.478	12.442± 1.817	8.413± 1.949
Vehicle 5ml/kg	6.9± 0.6046	8± 1.687	7.2± 1.625	8.1± 1.828	8.8± 1.489	9.8± 2.065	10.2± 1.511	6.6± 1.108
ETHOSUXI MIDE 95 mg/kg	13.175 ±1.406*	13.092 ±1.756*	13.175 ±1.406*	13.092 ±1.756*	13.175±1. 406*	13.092 ±1.756*	13.175±1. 406*	13.092± 1.756*
sETHOSUXI MIDE 125 mg/kg	15.862 ±1.844*	12.8±1. 916**	15.862 ±1.844*	12.8±1. 916**	15.862±1. 844*	12.8±1. 916**	15.862±1. 844*	12.8± 1.916**
ETHOSUXI MIDE 155 mg/kg	15.961 ±1.589**	15.26± 1.518***	15.961 ±1.589**	15.26± 1.518***	15.961±1. 589**	15.26± 1.518***	15.961±1. 589**	15.26± 1.518***
ETHOSUXI MIDE 190 mg/kg	16.188 ±1.061 *	15.383 ±1.273 **	16.188 ±1.06 1*	15.383 ±1.273 **	16.188±1. 061*	15.383 ±1.273 **	16.188±1. 061*	15.383± 1.273**
ETHOSUXI MIDE 220 mg/kg	16.1±1. 356*	15.35± 1.042**	16.1± 1.356*	15.35± 1.042**	16.1±1.356*	15.35± 1.042**	16.1±1.356*	15.35± 1.042**

* denotes p<0.05 when compared with Normal rats by students unpaired 't' test.

** denotes p<0.01 when compared with Normal rats by students unpaired 't' test.

*** denotes p<0.001 when compared with Normal rats by students unpaired 't' test.

Table 2: Comparison of different doses of Ethosuximide in neuropathy induced rats (Vincristine treated rats)

	Day second			Day sixth			Day eighth			Day tenth		
	HPM	**TIT**	**ADM**	**HPM**	**TIT**	**AD M**	**HPM**	**TIT**	**ADM**	**HPM**	**TIT**	**ADM**
VINCRSIT INE 50mcg/kg	4.6± 0.339	8.5±1.765	5.4± 0.67	6.1± 0.6403	5± 0.7601	10.5± 1.003	6.6± 0.733	4.3± 0.472	11.1± 1.32	8.7± 1.359	3.4± 0.4761	12.3± 0.943
ETHOSUX IMIDE 95 mg/kg	14.14± 0.622***	12.14± 0.65	12.28 ± 0.485	14.14 ± 0.622***	12.14 ± 0.65***	12.28± 0.485	14.14± 0.622***	12.14± 0.65***	12.28 ± 0.485	14.14± 0.622**	12.14± 0.65***	12.28± 0.485
ETHOSUX IMIDE 125 mg/kg	14.33± 0.956***	14.66± 0.785**	11.83± 0.33***	14.33 ± 0.956***	14.66 ± 0.785***	11.83± 0.33	14.33± 0.956***	14.66± 0.785***	11.83 ± 0.33	14.33± 0.956**	14.66± 0.785***	11.83± 0.33
ETHOSUX IMIDE 155 mg/kg	14.66± 0.853***	14.66± 1.13**	10± 0.315***	14.66 ± 0.853***	14.66 ± 1.13***	10±0.315	14.66± 0.853***	14.66± 1.13***	10± 0.315	14.66± 0.853*	14.66± 1.13***	10± 0.315*
ETHOSUX IMIDE 190 mg/kg	15.33± 0.763***	14.33± 0.956**	9.33± 0.511***	15.33 ± 0.76***	14.33 ± 0.956***	9.33± 0.511	15.33± 0.763***	14.33± 0.956***	9.33± 0.511	15.33± 0.763***	14.33± 0.956***	9.33± 0.511*
ETHOSUX IMIDE 220 mg/kg	15.16± 0.915***	18± 0.66***	8.16± 0.451**	15.16 ± 0.91***	18± 0.66*	8.16± 0.451*	15.16± 0.915***	18± 0.66***	8.16± 0.451*	15.16± 0.915***	18± 0.66***	8.16± 0.451***

* denotes $p<0.05$ when compared with Normal rats by students unpaired 't' test.
** denotes $p<0.01$ when compared with Normal rats by students unpaired 't' test.
*** denotes $p<0.001$ when compared with Normal rats by students unpaired 't' test.

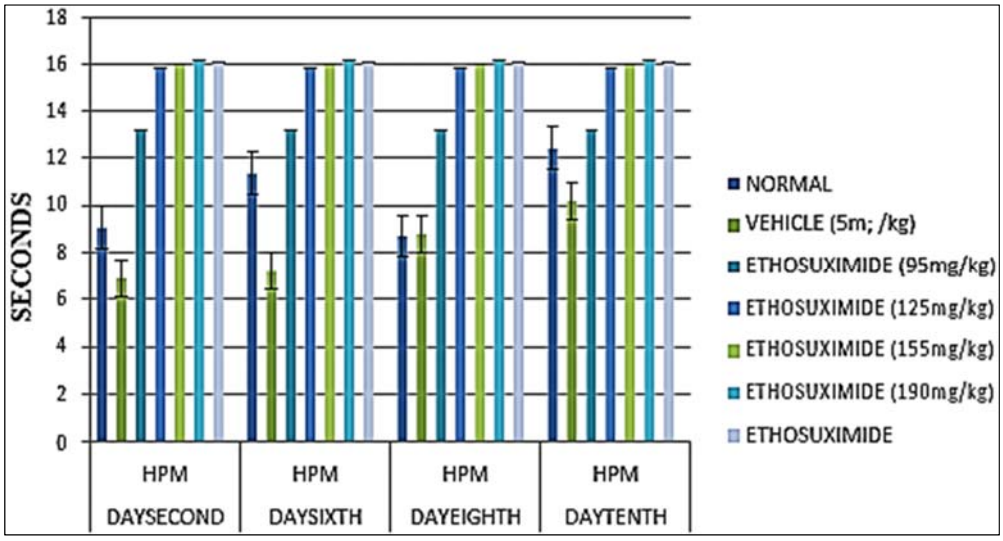

Fig 1: Comparision of different doses of ethosuximide In Normal Rats in Hot Plate method

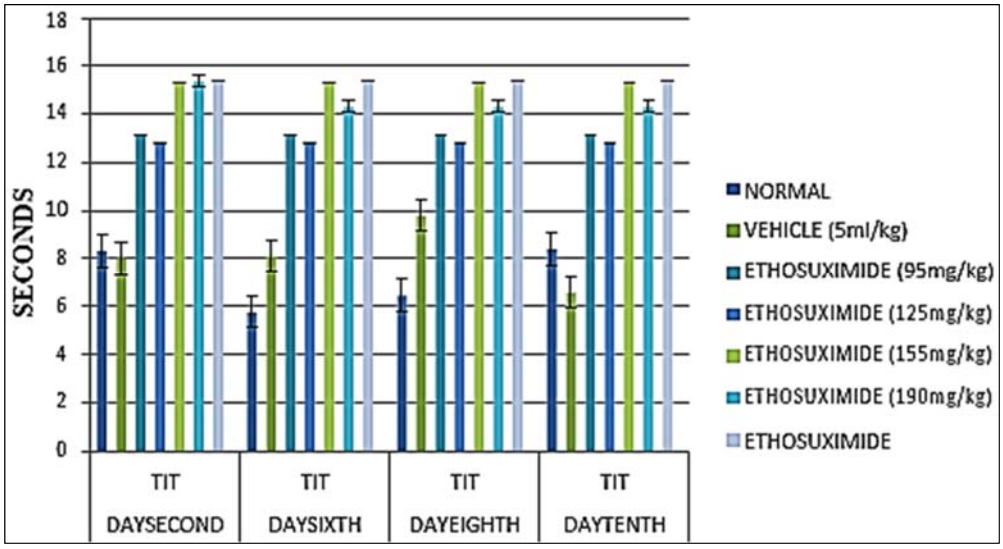

Fig 2: comparision of different doses of ethosuximide in normal rats in tail immersion test

Ethosuximide (95 mg/kg, 125 mg/kg, 155 mg/kg, 190 mg/kg &220 mg/kg, i.p) produced significant analgesia in HPM in normal rats on all days.

Ethosuximide (95 mg/kg, 125 mg/kg, 155 mg/kg, 190 mg/kg &220 mg/kg, i.p) produced significant analgesia in TIT in normal rats on all days. (see table 1& figure1 &2)

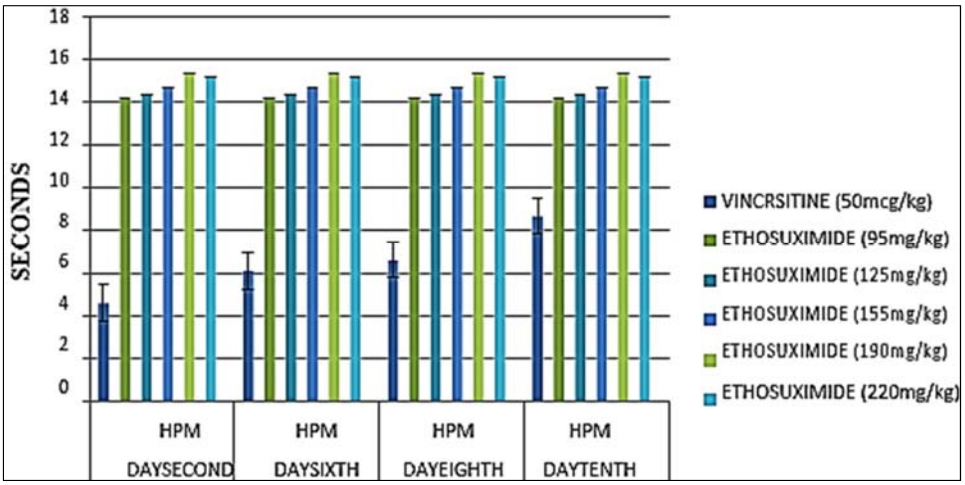

Fig 3: Comparision of different doses of ethosuximide with vincristine treated rats (neuropathy group) in hot plate method (HPM)

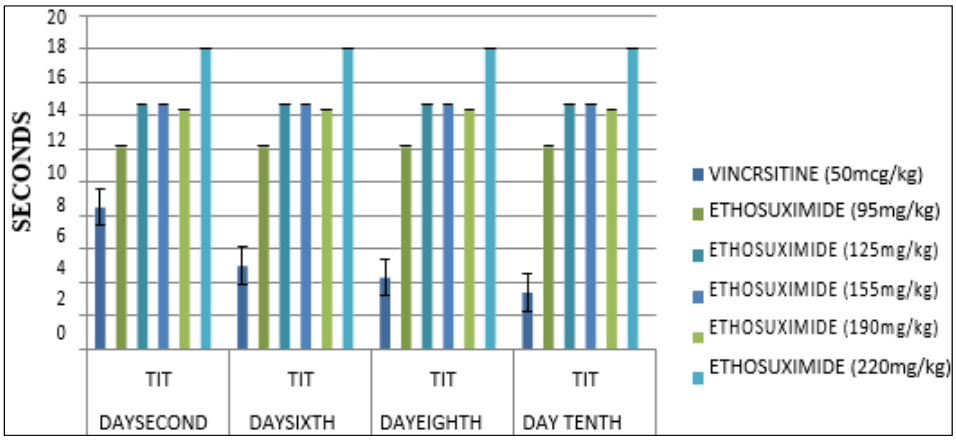

Fig 4: Comparision of different doses of ethosuximide with vincristine treated rats (neuropathy group) in coldtail immersion test (TIT)

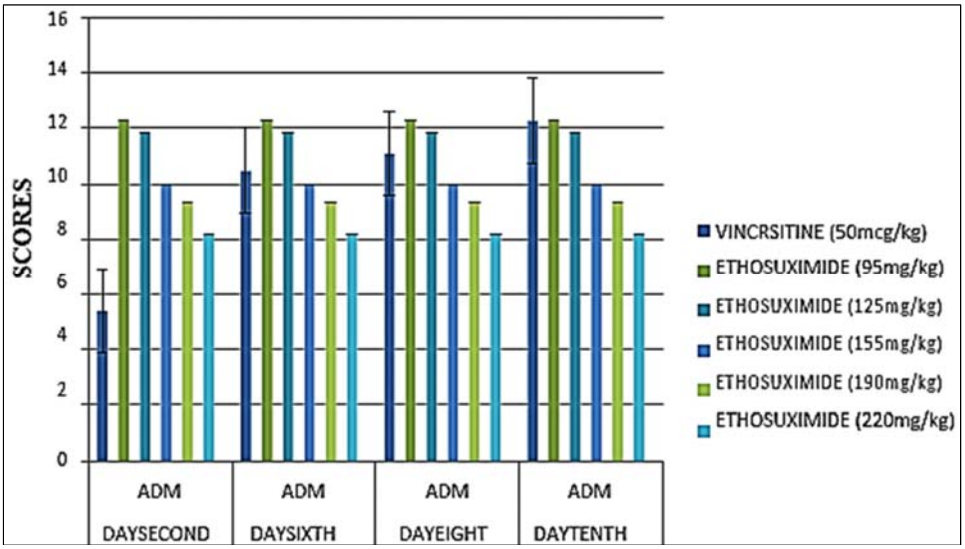

Fig 5: Comparision of different doses of ethosuximide with vincristine treated rats (neuropathy group) in acetone drop method (ADM)

Ethosuximide (95 mg/kg, 125 mg/kg, 155 mg/kg, 190 mg/kg &220 mg/kg, i.p) produced significant analgesia in HPM & TIT in Vincristine treated (neuropathic group) rats on all days. (see table 2 & figure 3&4) Ethosuximide in all doses reduced cold Allodynia significantly on second day in all doses. (see table 2 & figure 5)

5. Discussion

In our study Double distilled water, which was used as vehicle for dissolving different drugs (1ml/200 gm), did not produce any analgesia in Normal and Neuropathic group of Rats.

In our study, we found out that Ethosuximide (95 mg/kg, 125 mg/kg, 155 mg/kg, 190 mg/kg &220 mg/kg, i.p) produced significant analgesic effect in hot algesia (Hot Plate Method [HPM]) in Normal rats on all days i.e. 2nd, 6th, 8th and 10thday.

It produced significant analgesia in cold algesia methods (Tail Immersion Test [TIT]) on all days i.e. 2nd, 6th, 8th and 10th day.

It was not effective in Acetone Drop Method (ADM) since there was no Allodynia.

This Nociceptive effect of Ethosuximide in Models of acute pain is similar to that of Barton *et al*., 2005 [15] and to Todorovic *et al*., 2001 [16]. It differs from Dogrul *et al*., 2003[17] who have reported neither Ethosuximide nor mibefradil produced thermal antinociception in either the uninjured limb of sciatic nerve ligated rats or in sham operated rats.

The reasons for these differences in the acute antinociceptive Efficacies of T- type channel blockers are not entirely apparent, But may be related to the differences in the pain models, doses, Or routes of administration used in each study.

We found that Ethosuximide (95 mg/kg, 125 mg/kg, 155 mg/kg, 190 mg/kg &220 mg/kg, i.p) alleviated Thermal Hyperalgesia (HPM &TIT) in Neuropathic rats thus producing significant analgesia on all days of examination i.e. 2nd, 6th, 8th &10th days. Thus, it is much effective in relieving Hyperalgesia in neuropathic conditions.

It also relieved Cold Allodynia significantly on 2nd day and on 10th day in our study. Our finding is similar to Flatters *et al*., 2004 [18] who have also reported that Ethosuximide (i.p. 450 mg/kg) elicited a near complete reversal of mechanical Allodynia /Hyperalgesia. Our results are similar to (for neuropathic pain but in different model) to Hamidi *et al*., 2012 [19].

6. Conclusion

Ethosuximide in doses used produces significant analgesia in Normal rats.

It significantly reduces Thermal Hyperalgesia in Vincristine induced neuropathic rats. It is also effective in alleviating cold Allodynia in neuropathic rats.

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