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To Evaluate Analgesic Activity of Ethosuximide in Normal Rats and Neuropathic Pain Induced Rats

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Abstract: A novel therapeutic use of Ethosuximide has come to light with the findings of powerful analgesic effects in experimental models as well as in humans.The analgesic effects of Ethosuximide were explored in various nociceptive models. Following intraperitoneal administration, ethosuximide dose-dependently reversed chemotherapy induced peripheral neuropathic pain and capsaicin-induced mechanical Allodynia, and produced antinociceptive effects in the rat-tail flick reflex test in male rats. Analgesia Produced by Ethosuximide is as good as that Produced by Gabapentin in Acute Pain models. Analgesia produced by extract of Ocimum sanctum is as good as that Produced by Ethosuximide, when compared with these drugs individually in acute pain models. In Neuropathic Pain induced by Ethosuximide Produces Significant Analgesia in Thermal Hyperalgesia Models and significantly reduces Cold Allodynia. The Ethosuximide in neuropathic pain relievers Thermal Hyperalgesia as well as cold Allodynia.
Study Design: Observational Study
Keywords: Analgesic, Ethosuximide, Nicorandil, Neuropathic pain, Rats.

INTRODUCTION

Neuropathic Pain causes positive and negative symptoms and can occur spontaneously or following a provoking stimulus. Negative symptoms-damage to the nervous system causes loss of sensibility where the degree of loss approximates with the severity of impairment.

Positive symptoms-minority of cases present with different types of pain and dysesthesias (spontaneous or provoked unpleasant abnormal sensation). Spontaneous pain (continuous or intermittent) is commonly described as sharp stabbing or burning.

Pain provoked by a stimulus is characterized by Hyperalgesia (increased pain induced by non-painful stimuli) and Allodynia (pain caused by non-painful stimuli) that results from mechanical thermal or chemical stimulations [1].

Regardless of the cause, characteristic clinical symptoms of neuropathic pain include the feeling of pins and needles burning shooting and/or stabbing pain with or without throbbing and numbness [2-4].

MATERIALS & METHODS

The study was carried out at research Laboratory, Department of Pharmacology, NSCB Medical College, Jabalpur (M.P.), For the study of the evaluation and Comparison of Analgesic Activity of Ocimum Sanctum Extract with Analgesic Activity of Gabapentin, Nicorandil, Ethosuximide in Animal Model of Neuropathic Pain, following materials and methods were used:

DRUGS AND CHEMICALS USED

Drugs	Manufacturer
Ethosuximide (Powder) (Analytical Grade)	Sigma Aldrich Chemie, USA Product of Netherland

Methods used

Induction of Neuropathic pain by Vincristine

Vincristine (50µg/kg i.p. OD) was administered in each rats of the Group for 10 consecutive days, which lead to Peripheral neuropathy [5].

Behavioral Examinations

Paw cold Allodynia (Acetone Drop Test)

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 was recorded with an arbitrary minimum value of 0.5s and a maximum of 20s[5].

Each rat was sprayed 100µL of Acetone on each Hind Paw. The rat was observed for No licking; quick withdrawal; Prolonged withdrawal OR repeated Flicking; Repeated flicking WITH Licking of the paw. The duration was measured in seconds using stopwatch to finish the above mentioned observations within the cut off time of 20 seconds. The Time was measured by another assistant and observations were done by the main researcher.

After 10 minutes of gap, again each rat was sprayed 100µL of Acetone on each Hind Paw. The rat was observed for No licking; quick withdrawal; Prolonged withdrawal OR repeated Flicking; Repeated flicking WITH Licking of the paw. The duration was measured in seconds using stopwatch to finish the abovementioned observations within the cut off time of 20 seconds. The Time was measured by another assistant and observations were done by the main researcher. Same procedure as mentioned above was repeated for the third time after a gap of 10 minutes on each rat.

The final scoring for all the three ADM procedure done on each rat of the respective groups was done according to method described by Choi Y and Flatters & Bennet[6,7].

STATISTICAL ANALYSIS

All the results expressed are mean± standard error of mean (Mean±S.E.M). The means of different groups are tested for their differences with Student's unpaired't' test. The statistical Analysis was done using Graph Pad Prism Software. $p<0.05$ is considered significant and $p<0.01$ is considered very significant and $p<0.001$ is considered to be extremely significant will be considered significant.

OBSERVATION & RESULTS

ETHOSUXIMIDE IN NORMAL HEALTHY RATS

Ethosuximide (100mg/kg,i.p.) was administrated in ten healthy albino rats (150-250gms). No neuropathy was induced. Following behavioral tests for evaluation of analgesic activity were performed. The observations and results are as following:-

Thermal Algesia Test (Hot Plate Method [HPM])

Cut off time for all rats to lift or lick their hind paw was twenty seconds (20 sec.)

The Mean ± SEM for day 2^{nd} and 4^{th} is 12.40±1.00, 11.80±0.80 seconds, respectively.

The results are shown in Table No.1 & Figure No.1

On day 2 the difference of these means with normal rats was statistically significant (p value=0.0001, $p<0.001$).

On day 4 the difference of these means with normal rats was statistically significant (p value =0.0001, $p<0.001$).

See Table No. 2 & Figure No.2

Thus, Ethosuximide administered in normal Rats has analgesic activity in HPM.

On day 2 statistically not significant (p value=0.4886, $p>0.05$).

On day 4 statistically not significant (p value=0.6200, $p>0.05$).

Table-1: Results showing evaluation of analgesic activity of Ethosuximide (100mg/kg, i.p.) when administrated in normal rats

Test	Day 2(seconds)	Day 4(seconds)
HPM (seconds)	12.40±1.00	11.80±0.80
TIT (seconds)	8.60±0.76	10.10±1.59
ADM (scores)	0	0

HPM= Hot Plate Method; TIT= Tail Immersion Test; ADM= Acetone drop method

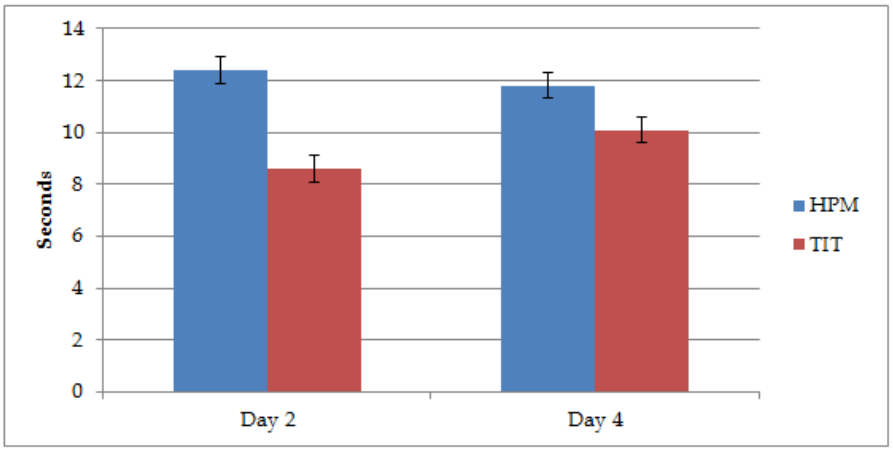

Fig-01: Bar diagram showing analgesic activity of ethosuximide (100mg/kg,i.p.) when administrated in normal rats in hot plate method and tail immersion test

Table-2: Comparison of paw withdrawal latencies (analgesic activity) of ethosuximide treated normal rats with paw withdrawal latencies of normal group (Group 1) in hot plate method

Test	Day 2(seconds)	Day 4(seconds)
Normal	5.10±0.53	5.30±0.53
Ethosuximide	12.40±1.00***	11.80±0.80***

*** denotes p value < 0.001 which is extremely significant when compared with Normal Group (Group 1) by student unpaired 't' test.

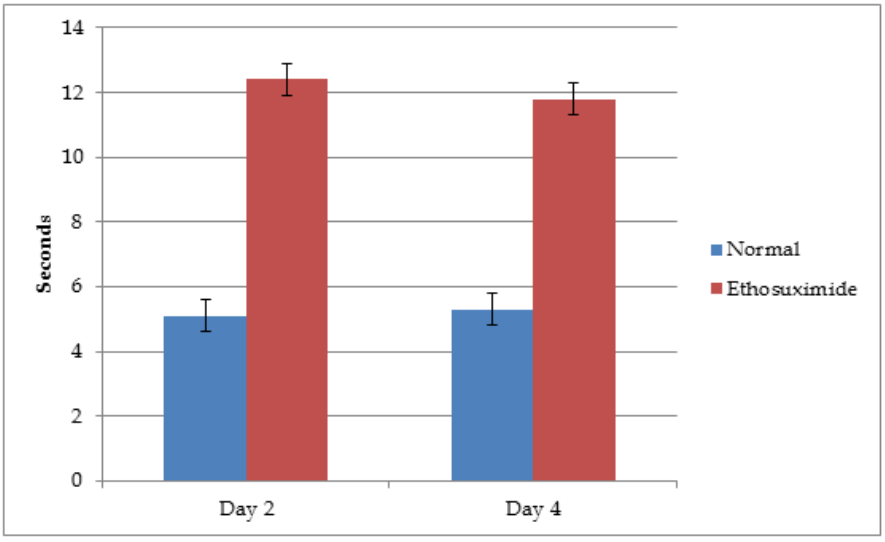

Fig-2: Bar diagram showing paw withdrawal latencies (analgesic activity) of ethosuximide treated normal rats with paw withdrawal latencies of normal group in hot plate method

DISCUSSION

In our study, we found out that Ethosuximide (100 mg/kg, i.p) produced significant analgesic effect in Hot and cold algesia methods (Hot Plate Method [HPM], Cold Tail Immersion Test [TIT]; Acute Thermal Pain Models) in Normal rats. 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.*[8] and to Todorovic *et al*. [9] It differs from Dogrul *et al.*[10] 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.

Our finding is similar to Flatters *et al*. [11] who have also reported that Ethosuximide (i.p. 450 mg/kg) elicited a near complete reversal of mechanical

Allodynia /Hyperalgesia. In their study Repetitive dosing with Ethosuximide (100 or 300 mg/kg daily for 3 days) showed a dose-related consistent reversal of mechanical allodynia/hyperalgesia with no evidence of tolerance. We have not given the Ethosuximide daily but have administered it on second and fourth day after neuropathy. We found that after administration on each of these days, Ethosuximide alleviated Thermal Hyperalgesia and Cold Allodynia.

They further noted that, Ethosuximide (300 mg/kg i.p) also reversed paclitaxel-induced cold Allodynia and Vincristine-induced mechanical Allodynia/ Hyperalgesia. Our results are similar to (for neuropathic pain but in different model) to Hamidi *et al*. [12] who have reported that, Ethosuximide (100,200, 300 mg/kg i.p) reduces cold and mechano Allodynia and thermal Hyperalgesia in the chronic constriction injury model of neuropathic pain. Dorgul *et al*. [13] have also reported that systemic Ethosuximide produced dose dependent blockade of both tactile and thermal hypersensitivities in nerve injured rats.

From the above it can be said that Ethosuximide acts as analgesic in Different neuropathic Pain conditions. Our study also supports that at doses of 100 mg/kg, Ethosuximide, when given by systemic route, relieves Hyperalgesia and Allodynia of Vincristine induced Neuropathy. Calcium dysregulation in Vincristine and paclitaxel induced neuropathic pain has been established, Ethosuximide a selective T type VGCC, Thus might play a role in Vincristine induced neuropathy. Further Clinical Studies are required to document efficacy of Ethosuximide in Neuropathic conditions in Humans.

CONCLUSION

Analgesia Produced by Ethosuximide is as good as that Produced by Gabapentin in Acute Pain models. Analgesia produced by extract of Ocimum sanctum is as good as that Produced by Ethosuximide, when compared with these drugs individually in acute pain models. In Neuropathic Pain induced by Ethosuximide Produces Significant Analgesia in Thermal Hyperalgesia Models and significantly reduces Cold Allodynia. The Ethosuximide in neuropathic pain relievers Thermal Hyperalgesia as well as cold Allodynia.

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