

## Original Research Article

# Comparative evaluation of phasic and chemical antinociceptive action of a conventional and a novel anticonvulsants in experimental models of tail flick and formalin test

Saurabh Kansal<sup>1\*</sup>, Rohan Sirohi<sup>2</sup>, Ruchika Agarwal<sup>1</sup>

<sup>1</sup>Department of Pharmacology, <sup>2</sup>MBBS Student, Subharti Medical College, Meerut, Uttar Pradesh, India

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**\*Correspondence:**

Dr. Saurabh Kansal,

E-mail: kansalsaurabh513@gmail.com

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### ABSTRACT

**Background:** Some antiepileptic drugs have been shown to be clinically efficacious in treatment of neuropathic pain and are being used by clinician.

**Methods:** This study determined the analgesic effect of gabapentin (a conventional anticonvulsant) and levitiracetam (a novel anticonvulsant) in rats in different types of acute and chronic nociceptive test like tail flick and formalin test and compared its potency with a conventional non opioid analgesic diclofenac.

**Results:** Per oral administration of gabapentin produced no any marked effect on early phase response of formalin test but significantly suppressed the late phase response while levitiracetam produced no any type of significant effect in both phases. In tail flick test gabapentin as well as levitiracetam produced no any significant analgesic effect while diclofenac produced significant reduction of pain in tail flick test as well as in both phases of formalin test.

**Conclusions:** Thus, we have observed that gabapentin produced antinociception in chronic pain as second phase of formalin test reflects chronic inflammatory pain while levitiracetam did not produce any type of antinociceptive effect as it could not suppress the pain significantly in both tail flick and formalin test.

**Keywords:** Diclofenac, Gabapentin, Formalin test, Levetiracetam, Nociception, Tailflick test

### INTRODUCTION

Pain as a sensation and feeling, is a known entity from antiquity. According to the IASP- "pain is an unpleasant, sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage".<sup>1</sup>

Pain could be acute or chronic in nature. Acute pain is short lasting and easy to manage while chronic pain is that pain which persists beyond the usual course of injury or diseases or reoccurs in every few months or years. Pathologically chronic pain state could be inflammatory or neuropathic. Inflammatory pain is due to chronic inflammation that is increased by pressure, but

neuropathic pain occurs due to involvement in alteration in nervous system function or reorganization of nervous system structure and are non adaptable. NSAIDs and opioids are the most potent and commonly used group of established analgesic drugs in treatment of pain, but there use is associated with a greater degree of adverse drug reactions and abuse liability.<sup>2</sup>

The anticonvulsants carbamazepine and gabapentine are now established drugs for trigeminal neuralgia and postoperative pain as non conventional analgesics.<sup>3</sup> Other anticonvulsants are also being tried as newer unconventional analgesic drugs that are expanding day by day.

There are no comparable data available, whereby these drugs could be compared simultaneously for their analgesic activity in suitable animal models of acute and chronic pain, although there is some consistency in their effects as far as neuropathic animal pain models are concerned.

So, the present study was planned to verify the effects of novel anticonvulsant i.e. gabapentin along in common acute (tail flick test) and chronic inflammatory (formalin test) pain models. Thus, we examined the antinociceptive effect of gabapentin (a newer anticonvulsant) in animal models of pain and compared its antinociceptive effects also with conventional nonopioid analgesic diclofenac.

## METHODS

This study has been carried out in department of pharmacology, HIMS, Dehradun over a period of 12 months for evaluation of analgesic effects in animals. This study has been approved by ethical committee of HIMS.

### *Animals*

An adult albino rats of either sex, weight of 150-200gm have been utilized for these experiments.

### *Drugs*

The following drugs have been used to evaluate their antinociceptive effects in each group of 6 animals, given p.o. 1hr before the experimentations. There has been a control group of 6 animals, run simultaneously, and given saline/vehicle p.o. as per the experiment. All the experiment was done at the same time in the morning hours on all days of experimentation. Gabapentin 500mg/kg<sup>1</sup>, levitiracetam 60mg/kg<sup>4</sup>, diclofenac 5mg/kg<sup>5</sup> doses were used in this study.

Commercial preparations of these drugs have been used. Levitiracetam (Ranbaxy Lab Ltd., Ponta, Goa) has been dissolved in saline as it is a water-soluble drug. Gabapentin (Sunpharma, Dadra, New Delhi) and control drug diclofenac (Novartis India Ltd. Pune, India) were suspended in 5% acacia and double deionized water both drugs were administered per oral by gavage in a volume of 1.0ml/kg in rats.<sup>6</sup>

### *Procedures: for antinociceptive evaluation*

#### *Radiant heat method*

The tail flick test by radiant heat method using analgesiometer was carried out to study antinociception. Each animal was placed in that manner so the proximal third of the tail of the animal was laid across nicrome wire coil, which was heated by the passage of an electric current. For about 6 seconds the reaction of the animal has been observed. Rats with reaction time for more than

6seconds have not been used in the test. The tests compounds have been administered per orally. The animals have been subjected to the same testing procedure after 0, 60, 90, and eventually 120minutes for each individual animal. The cut off time has been 10seconds.<sup>7,8</sup>

#### *Formalin test*

The formalin test has been used as the model of chronic inflammatory pain. Formalin has been characterized by the occurrence of two characteristic phases of increased pain sensitivity in rats. The first phase was of 0-15 minutes and phase 2 was of 45-75 minutes. Rat has been administered 0.05ml of 10% formalin into the dorsal portion of the front paw. The test drugs have been administered orally and scored according to a pain scale. Pain has been quantified by counting the incidence of spontaneous flinches, shakes and jerks of the formalin injected paw. Analgesic response or protection has been indicated if both paws are resting on floor with no obvious favouring of injected paw.<sup>7,8</sup> Treatment group was compared with appropriate control groups using "student t-test

## RESULTS

The present study was conducted in the Department of Pharmacology, Himalayan Institute of Medical Sciences, Swami Ram Nagar, Dehradun with the objective to experimentally evaluate the analgesic effect of novel antiepileptic gabapentin and one analgesic as positive control i.e. diclofenac.

For each set of experiment, six adult healthy Albino rats of either sex each were used for both experimental drugs. Gabapentin (50mg/kg p.o.), levitiracetam (60mg/kg) and diclofenac (5mg/kg) given 1 hr before the experimentations.

#### *Radiant heat test*

During the evaluation of antinociceptive effect in tail flick radiant heat test, only diclofenac produced significant increase in post drug latency at 120mins after drug administration. Experimental antiepileptic drug gabapentin and levitiracetam did not change tail flick latency over a period of 120min after administration of drug (Table 1).

#### *Formalin test*

The formalin test has been used as a model of tonic and inflammatory pain. Formalin has been characterized by two characteristic phases of increased pain sensitivity in rats. The first phase is of 0-15minutes denoting acute pain and phase 2 is of 45-75minutes denoting chronic inflammatory pain. Number of raising foot (LR) licking and biting (LB) were measured for the two phases as end points (Table 2, Figure 1).

**Table 1: Effects of experimental drugs and positive controls (diclofenac) on tail flick latency (in seconds) in radiant heat test over a period of 120min of drugs/vehicle (ns) p.o. administration in albino rats.**

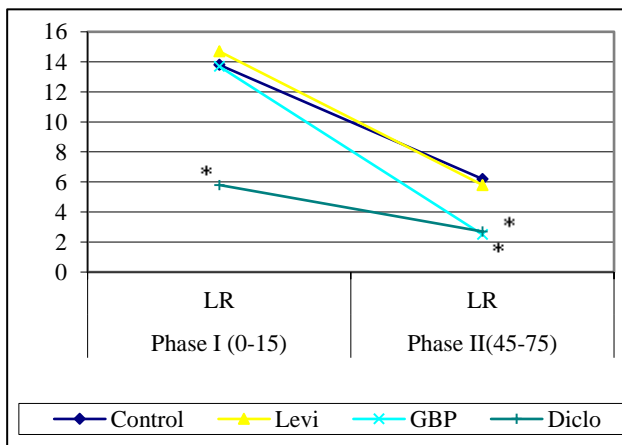
Group	No. of Albino rats	Dose and route of administration of drugs	Post drug latency (Mean±SE) (in seconds)			
			0 min	60 mins	90 mins	120 mins
Control	6	0.09% p.o	5.08±0.24	5.13±0.23	5.15±0.18	5.17±0.15
Diclofenac	6	5mg/kg p.o	5.15±0.26	5.30±0.25	5.42±0.25	5.55±0.23**
Gabapentin	6	50mg/kg p.o	5.15±0.24	5.20±0.22	5.22±0.18	5.28±0.14
Levitiracetam	6	60mg/kg p.o	5.12±0.23	5.17±0.22	5.18±0.18	5.20±0.14

\*\* p<0.05 vs control values at 0 min after diclofenac p.o administration, Albino rats producing a reaction time of <6sec were selected for experiment and a cut off time of 10sec was kept. Reading were taken at time interval 0,60,90 and 120min after drug/vehicle administration

**Table 2: Time effects of experimental drugs and positive controls (diclofenac) administered p.o. 1hr before on number of raising foot and licking and biting responses in Albino rats administered with dilute formalin (0.05ml of 10% in NS) in right forepaw on dorsal surface i.d.**

Group	No. of Albino rats	Dose and route of administration of drugs	Raising Foot (Mean±SE)		Licking and Biting (Mean±SE)	
			First Phase	Second Phase	First Phase	Second Phase
Control	6	0.09% p.o.	13.8±2.9	6.2±1.2	23.0±3.0	15.3±1.8
Diclofenac	6	10mg/kg p.o.	5.8±1.3*	2.7±0.5*	9.2±1.0**	6.3±0.7***
Gabapentin	6	50mg/kg p.o.	13.7±3.3	2.5±0.2*	22.0±3.3	6.8±1.4**
Levitiracetam	6	60mg/kg p.o.	14.7±2.8	5.8±1.1	21.3±2.5	13.5±2.1

\*p<0.05 vs control values, \*\*p<0.02 vs control values, \*\*\*p = 0.001 vs control values, Observation measured in 2 phases, 1st is 0-15min and 2nd is 45-75min

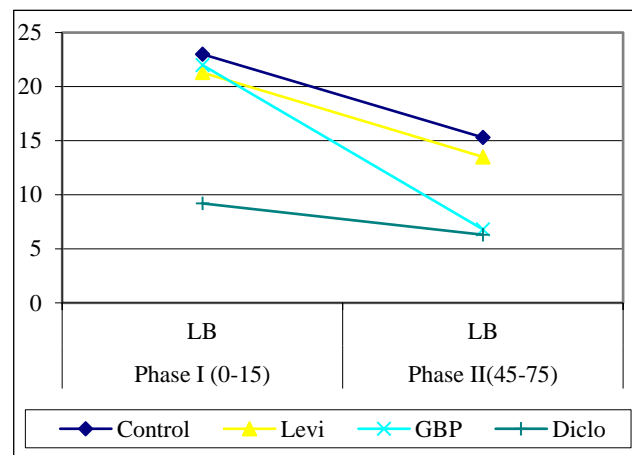


\*p<0.05 vs control values, \*\*p<0.02 vs control values, \*\*\*p = 0.001 vs control values, Observation measured in 2 phases, 1+ is 0-15min and 2<sup>nd</sup> is 45-75min

**Figure 1: Time effects of experimental drugs and positive control (diclofenac) administered p.o. 1hr before on number of raising foot responses in albino rats administered with dilute formalin (0.05ml of 10% in ns) in right forepaw on dorsal surface i.d.**

In the first phase of leg raising (LR) formalin test, positive control diclofenac produced significant decrease in leg raising while both experimental antiepileptic drugs

produced no any significant effect on leg raising in comparison to control values.



\*\*p<0.02 versus control values, \*\*\*p = 0.001 versus control values, Observation measured in 2 phases, 1<sup>st</sup> is 0-15min and 2<sup>nd</sup> is 45-75min

**Figure 2: Time effects of experimental drugs and positive control (diclofenac) administered p.o. 1hr before on number of licking and biting responses in Albino rats administered with dilute formalin (0.05ml of 10% in NS) in right forepaw on dorsal surface i.d.**

In the first phase of licking and biting (LB), positive control (diclofenac) again produced significant decrease ( $p < 0.02$ ) than control values while gabapentin and levitiracetam had no effect.

In the second phase of raising foot (LR) both diclofenac and gabapentin produced significant decrease ( $p < 0.05$ ) but not levitiracetam as compared to control values. In the licking and biting episodes of second phase also gabapentin and diclofenac exert significant effect ( $p < 0.02$ ) but levitiracetam did not produce any significant effect in comparison to control. Decrease observed in licking and biting (LB) with diclofenac was more ( $p = 0.001$ ) as compared to control values than with experimental antiepileptic drug ( $p < 0.02$ ) versus control values (Figure 2). To conclude, the present study investigated analgesic property of gabapentin and levitiracetam in both animal models of pain. Diclofenac found significantly effective in tail flick test. While In formalin test both diclofenac and gabapentin produced significant effect in phase 2 of formalin test. Levitiracetam did not produce any type of significant effect in both tests.

## DISCUSSION

The present study was done to evaluate the antinociceptive effect of conventional and novel antiepileptics i.e. gabapentin and levitiracetam on different acute animal pain models i.e. phasic pain model (tail flick by radiant heat method) and tonic inflammatory pain model (formalin test) with the help of conventional analgesic drugs i.e. diclofenac which was used as positive control in rats.

Diclofenac is well established analgesic drug that showed significant antinociceptive effect in tail flick test when given orally (5mg/kg) in present study at 120min. It is in conformity with previous studies of diclofenac in which diclofenac showed analgesic effect in tail flick test while given 0.001-10.0mg/kg, body weight i.p.; 5mg/kg, i.v., 1-50microgm, i.c.v, 0.9-10microgm, i.t.<sup>9,10,5</sup>

In formalin test, diclofenac presently produced significant analgesic effect in both phase 1 and phase 2 pain which confirms to an earlier study in which diclofenac at a dose of 5, 10 and 20mg/kg, i.p. produced significant antinociceptive effect in both phases of formalin test.<sup>11</sup> Furthermore, diclofenac, 5mg/kg, i.v. had produced analgesic effect alone or in combination with opioid and pretreatment with local diclofenac, 25-200mg/paw in formalin test in the past.<sup>5,12</sup>

In our study in tail flick test no significant antinociceptive effect has been observed with gabapentin 50 mg/kg. This is in conformity with earlier studies in which gabapentin, 300 microgram, i.t. and 300mg i.c.v. produced no significant effect in tail flick test.<sup>12,13</sup>

In formalin test, in present study gabapentin, 50mg/kg, p.o. produced significant effect in phase 2 but not in phase 1 which is very similar to previous study in which gabapentin, 300mg i.t inhibited second phase flinching behavior significantly but not in phase one.<sup>14</sup> In another study gabapentin when given intraplantarly with either 6/60mcg had significantly reduced flinching behavior during phase 2, however phase 1 flinching behavior was unaffected.<sup>15</sup> Gabapentin in formalin test had produced a dose related inhibition of phase 2 with ED50 values of 22.9mg/kg, i.p, but not of phase 1 and it is also reported that gabapentin, 30mg/kg, s.c. and 100mg/kg, s.c. inhibited the late phases of nociceptive responses supporting present findings.<sup>16-18</sup>

In case of levitiracetam, 60mg/kg, p.o, in present study did not produce any significant effect in both animal pain models. The negative findings are also reported earlier where levitiracetam 100-500mg/kg, i.p. did not alter nociceptive reflex threshold in rats as determined by paw withdrawal latency to radiant heat and the pressure threshold for the tail flick response.<sup>4</sup> Also levitiracetam had also been reported to be devoid of analgesic properties and does not alter the electrophysiology of normal neurons.<sup>19</sup> Further in a study, levitiracetam did not show antinociception in both phases of formalin test when given in the dose range of 10-300mg/kg, i.p.<sup>20</sup>

The first and second phase of formalin test are generally believed to reflect excitation of peripheral afferent nociceptors and central sensitization, respectively consistent with previous reports gabapentin was found to attenuate second phase nociceptive behavior in the present study, suggesting a specific inhibition of central sensitization with alpha 2 delta binding in central neural axis of pain.<sup>21-23</sup>

## CONCLUSION

Evaluation of antinociception in acute and chronic pain models was done with the help of standard method of Tail flick and formalin test in Albino rats of either sex on novel anticonvulsant gabapentin. Diclofenac was used as positive control. Diclofenac as positive control was effective in both pain models. In tail flick test, which is the model of phasic pain using thermal stimuli with the help of hot nichrome wire, gabapentin and levitiracetam did not produce any significant antinociceptive effect. In formalin test, both the test drugs did not produce any significant effect on phase 1 denoting acute pain while in 2 phase which denotes prolonged inflammatory pain, only gabapentin produced significant antinociceptive effect.

Based on the present study it is concluded that newer anticonvulsant gabapentin, produces effects in chronic inflammatory pain models but does not affect acute nociception in animals. As formalin phase 2 chronic pain was relieved by both diclofenac and gabapentin. So, the

gabapentin could be effective in treatment of chronic inflammatory pain in humans also.

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*Conflict of interest: None declared*

*Ethical approval: The study was approved by the Institutional Ethics Committee*

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