ORIGINAL RESEARCH ARTICLE

EVALUATION OF MODULATORY ROLE OF GABAPENTIN AND MORPHINE IN ACUTE PAIN IN ALBINO RATS

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HOW TO CITE THIS ARTICLE:
Sridhar V. Maddikunta, Souris Kondaveti. "Evaluation of Modulatory Role of Gabapentin and Morphine in Acute Pain in Albino Rats". Journal of Evidence based Medicine and Healthcare; Volume 2, Issue 10, March 09, 2015; Page: 1464-1477.

ABSTRACT: The drugs morphine and gabapentin were tested upon the albino rats using two experimental methods- thermally and chemically induced pain (acute pain models). A brief review of literature on pain, its modulation mechanisms, classification of analgesics and their description, review of drugs used in this study and various methods of evaluation of analgesics has been described. This study evaluated and obtained results with morphine and Gabapentin individually and their combined effect, and compared their analgesic effects with the standard drug morphine. It was observed that the Gabapentin enhanced the analgesic effect of Morphine in the acute pain models. Gabapentin a well-tolerated novel antiepileptic with ant nociceptive effects and with a known safety profile could be considered as co adjuvant with Morphine in acute severe pain. However, this study gives an idea that by combining gabapentin with morphine, it might be possible to reduce the dose of Morphine to overcome its drug tolerance, dependence and respiratory depression, as both these drugs act through different pain modulating mechanisms. Further studies are needed to interpret the additive or synergistic effect of this combination.

KEYWORDS: Gabapentin, Morphine.

INTRODUCTION: Pain is a subjective phenomenon, and clinicians cannot reliably detect its presence or quantify its severity without asking the patient directly. The experience of pain includes the patient's emotional reaction to it and is influenced by many factors, including the patient's prior experience with pain, meaning given to pain, emotional stress, and family and cultural influences. Pain is an unpleasant sensation usually evoked by an external or internal noxious stimulus and is the most common symptom for which patients see a doctor. One of the greatest services doctors can do to their patients is to acquire skill in the management of pain in improving the overall quality of life. Opioids are still the drug of choice in severe pain treatment.[1] Its single dose in clinical use is limited by side effects such as sedation, nausea, constipation and respiratory depression.[2] In persistent pain opioid dose needs to be increased steadily due to poor response to pain secondary to the development of drug tolerance. On the other hand repeated administration of opioids leads to dependence. However certain types of pain may not respond to opioids.[3]

Drug dependence, tolerance and respiratory depression limit opioid use, which may be overcome by combination therapy. Therefore non-opioid analgesics are proposed to enhance opioid analgesic effect and also to attenuate side effects.[1]

Gabapentin, an alkylated amino butyric acid analogue, is a safe and well-tolerated anticonvulsant drug[4,5] which has demonstrated analgesic efficacy across a wide spectrum of pain

states.[6] It has been shown to be effective in animal models of neuropathic pain[7] like diabetic neuropathy,[8] trigeminal neuropathic disorders[9] and herpetic pain induced by viral infection in mice.[10] Also the antinociceptive effect of gabapentin was investigated in formalin induced inflammatory pain in rats[11] and postoperative pain.[12,13] Through these studies, it was demonstrated that gabapentin reduces pain transmission.[14] Therefore it was proposed that gabapentin might enhance morphine analgesia whenever a nerve injury has occurred. For example in rat model of neuropathy, gabapentin enhanced morphine analgesia.[15] Also some clinical studies showed that gabapentin can reduce morphine consumption after mastectomy[12] and after spinal surgery.[13]

Since there is role of GABA in pain modulation, studies have shown that Gabapentin increases brain extracellular GABA levels in both rats & humans.[16,17] In addition to enhancing GABAergic transmission, it has been hypothesized that Gabapentin modulates voltage-gated calcium channels, resulting in decreased excitatory neurotransmission in the dorsal horn of spinal cord, consistent with an inhibition of spinal nociceptive transmission.[18] The discovery of ·2· subunit of voltage-gated calcium channels as a high binding site for Gabapentin further supports the role of voltage-gated calcium channels in antinociceptive action.[19]

However, analgesic effect of gabapentin in acute pain model has not been studied well. So this study was performed to evaluate the analgesic effect of gabapentin alone and its combined effect with morphine in acute pain in albino rats. The analgesic effect was evaluated, using two methods:

1. Hot-plate method and
2. Writhing reflex using 0. 6% acetic acid.

AIMS AND OBJECTIVES:

1. To evaluate the analgesic effect of Gabapentin in thermally and chemically induced acute pain in albino rats of either sex.
2. To compare its analgesic effects with the standard drug morphine.
3. To evaluate combined analgesic effect of gabapentin and morphine in acute pain in albino rats.

MATERIALS AND METHODS: Materials and methods used to conduct the experimental study are:

a. Thermal stimulus (Hot-plate method).
b. Chemical stimulus (Writhing method).

1. Chemicals and Solutions:

Drugs:

- Morphine sulfate &,
- Gabapentin.
- Both the drugs were dissolved in normal saline and given subcutaneously.
- Acetic acid of 0. 6 % given intraperitoneally (for inducing writhing).
- Normal saline solution- control animals received subcutaneously.

2. Equipments: Eddy's Hot-plate (picture I), Insulin syringes, Tuberculin syringes, Bell jars and Glass beakers, Animal weighing balance, Animal cages, Cotton, Stop watch, Disposable needles, and Markers.

   Animals: Albino Rats.
   Weight: 150 -200 grams.
   Gender: either sex.
   Number of animals used: 48 Rats.

Each day of the experiment one animal from each group is taken up for experiment. All the rats were maintained on a standard 12hour light/dark cycle & with free access to food and water. Experiments were performed on light cycle at the same time in all the groups. Each animal was used once only and received drugs subcutaneously once only throughout the experiment.

Protocol has been approved by: The Institutional Animal Ethical Committee (No /IAEC-27/2010) and the guidelines for the animal care were strictly adhered to during the experimentation as recommended by committee for the purpose of control and supervision of experiments on animals (CPCSEA), Govt. of India. (The animals were procured from central animal house of Osmania Medical College).

Animal Groups:

Thermal stimulus- Hot-plate method:
The animals are grouped as follows: 4 groups of 6 animals each.
Total number of animals: 24.

Groups (drugs administered)

I group control (Normal saline-dose 1ml/kg body weight s. c.).
II group Std. Drug (Morphine- dose 3mg/kg body weight s. c.).
III group Test drug (Gabapentin-dose 90mg/kg body weight s. c.).
IV group combined effect of (Morphine & Gabapentin-dose 3mg + 90mg per kg body weight s. c.).

Chemical stimulus- writhing method: The rats in this method were divided into 4 groups of 6 animals in each, total number of animals: 24.

Induction of writhing by I. P. injection 0. 6% acetic acid.
I group control (Normal saline-dose 1ml/kg body weight s. c.)
II group Std. Drug (Morphine-dose 3mg/kg body weight s. c.)
III group Test drug (Gabapentin-dose 90mg/kg body weight s. c.)
IV group combined effect of (Morphine & Gabapentin-dose 3mg +90mg per kg body weight s. c.)

1. **Hot-plate method:** Thermal stimulus was employed by placing on Eddy's hot-plate maintained at temperature of about 55^0 centigrade to elicit paw licking response in rats by thermal method (picture II).

Exclusion Criteria: Albino rats weighing 150–200 grams were chosen for preliminary screening to exclude false positive tests/results; a dis-coordination test is carried out to exclude such possibilities. The animals are placed on a slowly rotating drum covered with mesh; the animals stable for >5 seconds included and the animals that fall off are considered as discordinated and were excluded from the experiment.

Rats are placed on hot plate maintained at 55^0 centigrade following administration of the drug under study, subcutaneously 15 to 20 minutes before.

Reaction time – time period between placing animal on hot plate and the response of licking of fore/hind paws noted for each animal.

Cut-off time – 30 seconds: if the reaction time exceeds more than 30 seconds it was assumed that analgesia has been produced and further delay might cause tissue injury.

The control (normal saline), the standard drug morphine, the test drug gabapentin and the combination of drugs morphine and gabapentin are administrated subcutaneously in four different groups of rats.

The measured quantities of drugs were given according to the body weight of the animals. The control group (I) received inj. Normal saline subcutaneously, the standard group (II) received inj. Morphine in the dose of 3 mg / kg body weight, test group (III) received inj. Gabapentin in the dose of 90 mg / kg body weight and group (IV) received inj. morphine 3 mg / kg BW plus inj. Gabapentin 90 mg / kg BW subcutaneously 15 minutes before placing on the hot-plate.

The animals were tested for latency period of analgesic effect in seconds and the results were noted at 0, 30, 60, 90, 120 minutes time intervals and tabulated.

Writhing test by chemical stimulus: Writhing was induced by intra-peritoneal administration of injection of 0. 6 % acetic acid at 1ml/kg of body weight. Animals that do not exhibit writhing within 30 seconds are discarded. In this method also the rats are divided into four groups. Group (I) control – received only inj. 0.6% Acetic acid intraperitonealy, group (II) received the standard drug inj. Morphine in the dose of 3 mg / kg BW, group (III) received test drug inj. gabapentin in the dose of 90 mg / kg BW and group (IV) received inj. Morphine 3 mg / kg BW plus inj. Gabapentin 90 mg / kg BW. 15 minutes before the induction of writhing by intra-peritoneal inj. 0. 6% acetic acid.

OBSERVATIONS: writhing or stretching syndrome characterized by a wave of contractions of the abdominal musculature followed by extension of the hind limbs.

Numbers of the writhes were recorded for the first 20 minutes. For scoring, a writhe is indicated by stretching of abdominal musculature with simultaneous stretching of at least one hind limb.

Formula for computing percentage of inhibition of writhings is

= Average writhes in the control group – Writhes in the test group x 100 Writhes in control group

The time period with the greatest percentage of inhibition of writhing is considered the peak time.

OBSERVATIONS AND RESULTS: The analgesic activity was based on

1. Increase in the mean reaction time of pain responses in rats to thermal stimulation by combined effect of Morphine and gabapentin in comparison to control and Morphine.
2. The percentage of inhibition of writhings to chemical stimulation in rats by the combined effect of Morphine and gabapentin in comparison to control and Morphine.

The different methods used to conduct the study: -

1. Hot-plate method (thermal stimulus).
2. Chemical method (chemical stimulus).

TABLES AND FIGURES: Table I showing rat paw licking response (picture II) to thermal stimulus in different groups at different time intervals showing mean reaction time.

Table II shows Mean percentage increase in reaction time to thermal stimulus (hot-plate method) in Rats at different time intervals in different groups.

Table III. Showing p values for rat paw licking response to thermal stimulus in hot-plate method, unpaired't' test was used for comparisons between different groups at different time intervals.

Table IV. Shows mean writhing episodes and percentage inhibition of writhings in comparison to control in rats to chemical stimulus.

Table V: Showing p values between different groups in inhibiting writhing episodes based on unpaired 't' test in chemical stimulus method.

Figures I and II shows bar charts and graphical representation denoting mean reaction time in hot-plate method in rats at different time intervals between different groups.

Figures III and IV shows mean percentage of latency period in rat paw licking response to thermal stimulus in hot-plate method between different groups.

Figure V shows percentage of decrease in number of writhing episodes in different groups of rats in chemical stimulus method.

STATISTICAL ANALYSIS: All the observations and results are presented in the form of mean, mean percentage change and standard error between groups -standard, test, and combined effect of standard and test drugs in comparison to control. Statistical analysis of data was performed using student 't' test to study the differences among the means. A p value < 0. 05 is considered significant, a P value <0.001 is considered highly significant.

DISCUSSION: The pain is defined as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage". Because of the complexity and multidimensional aspects of pain, the management of pain of severe type, remains the subject of many experimental and clinical studies. Use of Morphine and other opioids as "gold standard" in the treatment of severe pain is known to result in inadequate analgesia, drug tolerance, dependence and respiratory depression.[1,2,3] Therefore, as an alternative, use of non-opioids that produce analgesia when co-administered with morphine could have important clinical application.

Gabapentin which is known to be effective in different types of pain is proposed as a co-adjuvant to opioids especially morphine.[6,14]

The present study was to evaluate the analgesic effect of a novel antiepileptic, gabapentin with a known safety profile, in acute pain syndromes and compare it with the standard drug Morphine, a potent opioid analgesic and with control group in the thermally and chemically induced acute pain.

This experimental data demonstrated that the administration of Gabapentin alone and in combination with morphine induced analgesic effect in hot-plate method and writhing test.

The following parameters observed and the results were tabulated:

1. **Paw licking response:**
   Increase in mean reaction time at 30, 60, 90, 120 minutes to the thermal stimulus.
   Mean percentage increase in reaction time at 30, 60, 90, 120 minutes to the thermal stimulus.
2. **Writhing test:**
   Mean writhing episodes in the first 20 minutes after chemical stimulation and percentage of inhibition of writhings to the chemical stimulus.

Analysis of paw licking response: Statistical analysis of data was performed using unpaired 't' test for comparison between each group and to time interval.

As per the tables I, II, III and figures I, II, III and IV:

- At 'O' minutes showed the basal reaction time to thermal stimulus in all the groups.
- At 30, 60, 90, 120 minutes, Morphine showed an increase in the mean reaction time when compared at '0' minutes.
- The increase in the mean reaction time with Gabapentin was significantly less when compared to that with Morphine.
- The increase in the mean reaction time of the combined effect with Morphine plus Gabapentin when compared to that with Morphine alone at 30, 60 minutes was highly significant.
- The mean percentage increase in reaction time was highest in groups II, III, and IV at 60 minutes.

Analysis of writhing Reflex: As per the tables IV, V and fig. V

- The mean percentage inhibition of writhing reflex with Morphine was 60% when compared with the control group I was highly significant.
- The mean percentage inhibition of writhing reflex with Gabapentin being 45% when compared with the control group I was highly significant.
- The mean percentage inhibition of writhing reflex was 75% in group IV, when compared with control group I was highly significant. And when compared with Morphine at 30 minutes not significant but significant at 60 minutes post drug administration.

Based on the above findings:

- In Eddy's hot-plate method, the combined analgesic effect of Morphine and Gabapentin was more effective than Morphine alone in decreasing rat paw licking response.
- In the chemical method, combined analgesic effect of Morphine and Gabapentin also more effective than Morphine alone in decreasing rat writhing episodes.

CONCLUSION: The results obtained were analyzed and statistical significance evaluated using unpaired 't' test.

Finally based on the above study, conclusion drawn was:

- The combination of Gabapentin and Morphine demonstrated superior analgesic effect than Morphine alone in both the thermally and chemically induced acute pain models.

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Group	Reaction time in seconds				
	At '0'minutes	At 30 minutes	At 60 minutes	At 90 minutes	At 120 minutes
Group I	4. 3 ± 0.42	4. 3 ± 0.42	4. 30± 0.42	4. 3 ± 0.42	4. 3 ± 0.42
Group II	4. 5 ± 0.34	10. 2 ± 0.54	20 ± 0.73	17. 7 ± 1.58	10 ± 1.03
Group III	4. 3 ± 0.12	9. 7 ± 0.61	15 ± 0.86	12 ± 1.36	5. 33 ± 0.42
Group IV	4. 2 ± 0.28	15 ± 0.36	26. 7 ± 1.60	19. 33 ± 1.11	14 ± 1.4 6

Table 1: Hot-plate method

Groups drugs administered.
Group I normal saline.
Group II Morphine.
Group III Gabapentin.
Group IV (combined) Morphine + Gabapentin.

Group	At '0' minutes	At 30 minutes	At 60 minutes	At 90 s minutes	At 120 minutes
Group I	-	-	-	-	-
Group II	-	55. 74 %	78. 5 %	71. 69 %	50 %
Group III	-	53. 45 %	71. 33 %	58. 33 %	34. 78 %
Group IV	-	70 %	83. 88 %	74. 14 %	64. 28 %

Table 2: Hot-plate method- Mean percentage increase in reaction time

Groups drugs administered.
Group I normal saline.
Group II Morphine.
Group III Gabapentin.
Group IV (combined) Morphine + Gabapentin.

Groups	Time interval (min)			
	At 30 minutes	At 60 minutes	At 90 minutes	At 120 minutes
I vs II	0. 0001**	0. 0001**	0. 0006**	0. 0023*
I vs III	0. 0012*	0. 0001**	0. 0067*	0. 0409
I vs IV	0. 0001**	0. 0001**	0. 0001**	0. 0018*
II vs III	0. 5177	0. 0067*	0. 0302*	0. 0086*
II vs IV	0. 002*	0. 0129*	0. 4618	0. 1106
III vs IV	0. 001**	0. 0022*	0. 0060*	0. 0013*

Table 3: P values in hot-plate method byunpaired 't' test

*p values < 0. 05 significant.
**p values < 0. 001 highly significant.

Groups drugs administered.
Group I normal saline.
Group II Morphine.
Group III Gabapentin.
Group IV (combined) Morphine + Gabapentin.

Group	Mean writhing episodes	Standard Deviation	Standard Error	Mean percentage change
Group I	20	1. 78	0. 73	-
Group II	8	1. 78	0. 78	60 %
Group III	11	0. 89	0. 36	45 %
Group IV	5	1. 41	0. 58	75 %

Table 4: Writhing test – Mean percentage inhibition of writhing reflex

Groups drugs administered.
Group I normal saline.
Group II Morphine.
Group III Gabapentin.
Group IV (combined) Morphine + Gabapentin.

Comparison between groups	P values
I vs II	0. 0001**
I vs III	0. 0001**
I vs IV	0. 0001**
II vs III	0. 0043*
II vs IV	0. 0091*
III vs IV	0. 0001**

Table 5: P values in chemical method by unpaired 't' test

* P values < 0. 05 – Significant.
** P values < 0. 001- Highly significant.
Groups drugs administered.
Group I normal saline.
Group II Morphine.
Group III Gabapentin.
Group IV (combined) Morphine + Gabapentin.

Fig. 1: Hot-plate method - latency periods in seconds against different time intervals in different groups.

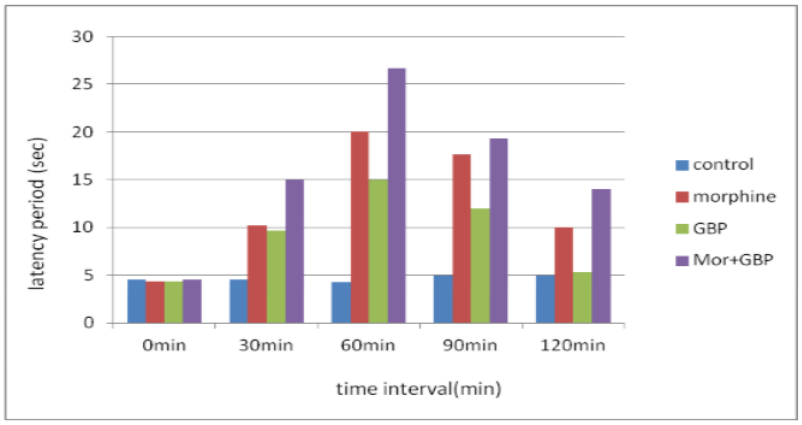


Fig. 1

Groups drugs administered.
Group I normal saline.
Group II Morphine.
Group III Gabapentin.
Group IV (combined) Morphine + Gabapentin.

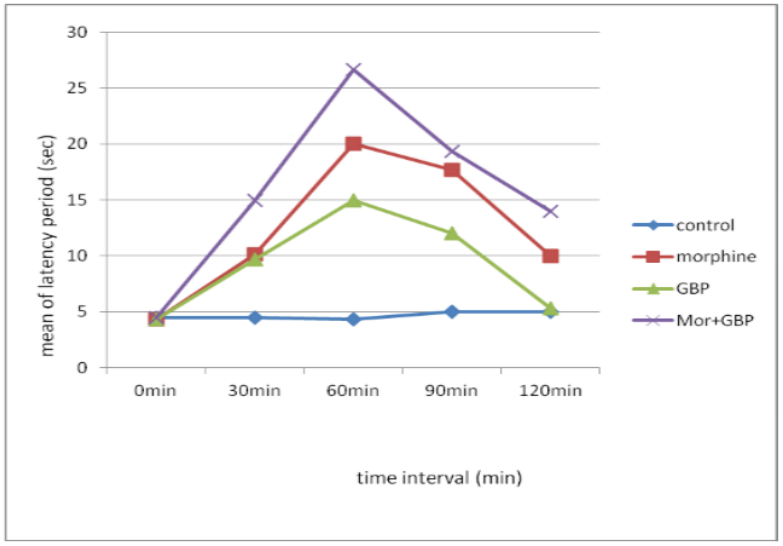


Fig. 2: Hot-plate method-mean latency periods

Groups drugs administered.
Group I normal saline.
Group II Morphine.
Group III Gabapentin.
Group IV (combined) Morphine + Gabapentin.

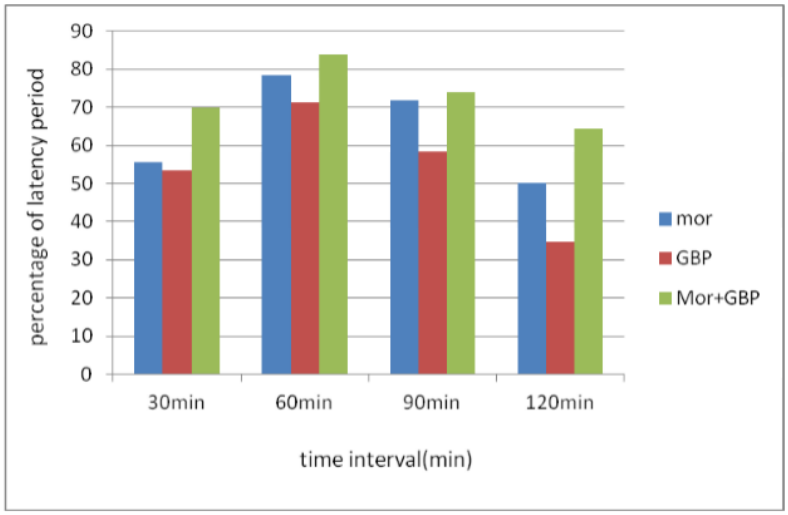


Fig. 3: Hot-plate method - percentage of latency periods

Groups drugs administered.
Group I normal saline.
Group II Morphine.
Group III Gabapentin.
Group IV (combined) Morphine + Gabapentin.

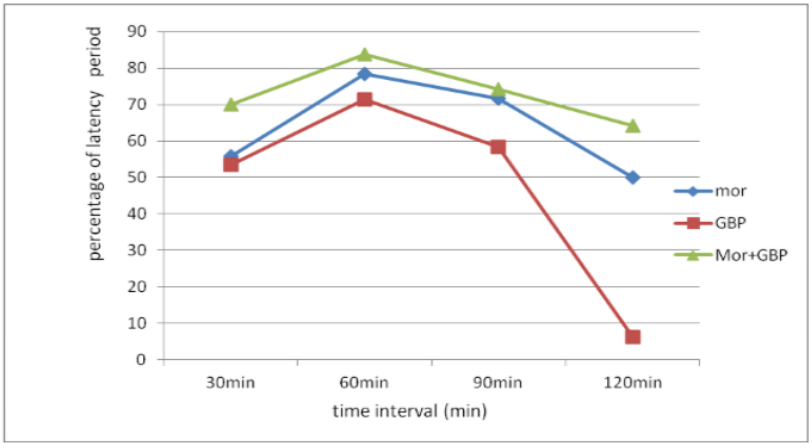


Fig. 4: Hot-plate method - percentage of latency periods

Groups drugs administered
Group I normal saline
Group II Morphine,
Group III Gabapentin
Group IV (combined) Morphine + Gabapentin.

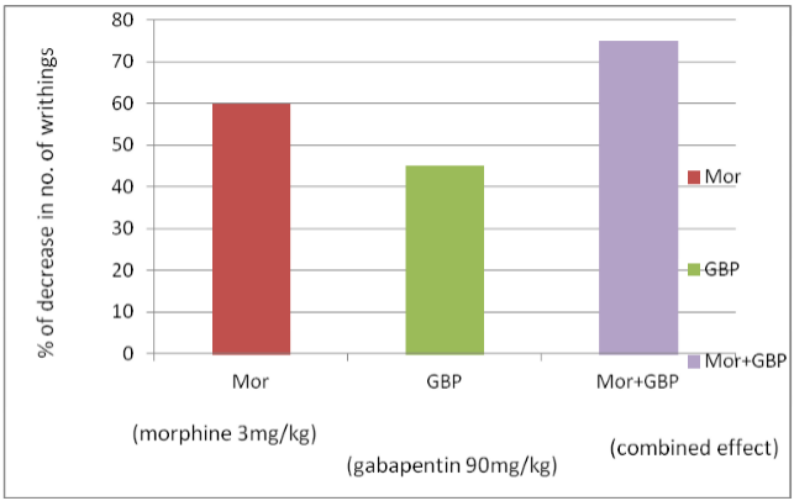
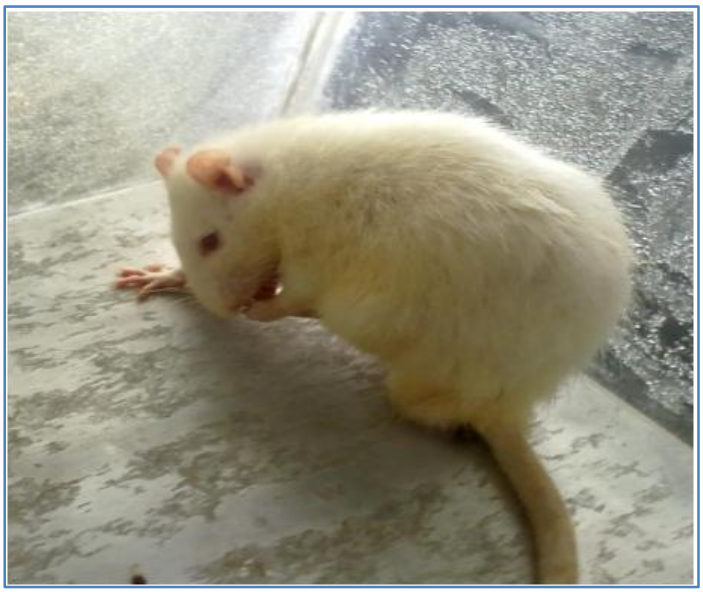


Fig. 5: Writhing test - percentage of inhibition of writhing

Groups drugs administered
Group I normal saline
Group II Morphine,
Group III Gabapentin,
Group IV (combined) Morphine + Gabapentin.

PICTURE I: SHOWING EDDY'S HOT-PLATE



PICTURE II: PAW LICKING RESPONSE WHEN PLACED ON EDDY'S HOT-PLATE

ACKNOWLEDGEMENT: I am much honored to thank my institution, Osmania Medical College, Thanks to Dr. Venkat Ramana, Dr. V. Prasanna and Dr. N. Vijaya.

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Date of Submission: 28/02/2015.
Date of Peer Review: 03/03/2015.
Date of Acceptance: 04/03/2015.
Date of Publishing: 05/03/2015.