

## Modulatory Role of Morphine and Gabapentin as Anti-inflammatory Agents Alone and on Co- administration with Diclofenac in Rat Paw Edema

S. Goyal\*, S. Verma and M.C. Gupta

*Department of pharmacology, Pt. BD Sharma PGIMS,  
Rohtak-124001 (Haryana), India*

\*Corresponding author e-mail: [drsaritagoyal@rediffmail.com](mailto:drsaritagoyal@rediffmail.com)

### ABSTRACT

**Objective:** To evaluate the anti-inflammatory effects of Morphine and Gabapentin alone and as adjuvant with diclofenac by using formalin induced paw edema model.

**Methods:** After acclimatization of one week, adult Sprague-Dawley rats were divided into two groups: control and treatment (n=6). Treatment group received diclofenac (10 mg/kg), gabapentin (150 mg/kg), morphine (15 mg/kg), gabapentin (150 mg/kg) + diclofenac (10 mg/kg) and morphine (15 mg/kg) + diclofenac (10 mg/kg), respectively. Paw edema was produced by injecting 0.2ml of 2% formalin subcutaneously on the dorsal surface of right hind paw. Animals received drug treatment 30 minutes before injection of formalin and paw volume was measured at 0, 30, 60, 120 and 240 minutes after formalin challenge with help of mercury plethysmometer.

**Results:** Both morphine and gabapentin alone and in combination with diclofenac caused a significant reduction ( $p < 0.01$ ) in rat paw edema when compared to group given saline only. Reduction in paw edema with gabapentin and diclofenac was significantly superior when compared with either drug alone.

**Conclusions:** Combination of gabapentin and diclofenac showed synergistic anti-inflammatory effect as compared to either drug alone or combination of morphine + diclofenac groups.

**Keywords:** Diclofenac, Gabapentin, Morphine, Inflammation.

### INTRODUCTION

Injury to living tissues causes a protective response in the form of inflammation which is body's defence

mechanism against the injurious agent.<sup>1</sup> This inflammatory reaction causes recruitment of cells like leukocytes and inflammatory

mediators at the injury site and leads to pain, redness and edema which can be very distressing.<sup>2</sup> Although inflammation is protective in some situations, if untreated, can lead to serious complications. Various drugs like Immuno-suppressants, corticosteroids, NSAIDs and anti-histaminics are being used, but the potential to cause side effects often limits their use. Because many of the inflammatory agents are only short acting, and often produce severe side effects, the need for new therapies continues.

Gabapentin, a structural analogue of neurotransmitter gaba-aminobutyric acid (GABA), apart from having antiepileptic properties, has shown promise in the treatment of chronic pain. It has exhibited antinociceptive effects against neuropathic pain and inflammatory hyperalgesia, which are hypothesized to be mediated by modulation of glutamate and GABA receptors and substance P neurotransmission.<sup>3,4</sup> It appears to be effective in reducing allodynia and hyperalgesia induced by inflammatory responses or nerve injury.<sup>5</sup>

Inflammation can modulate peripheral  $\mu$  opioid receptor function. Morphine, an opioid receptor agonist, when administered systemically, attenuates the inflammation and progress of the disease.<sup>6</sup> and can reduce inflammation-induced extravasation,<sup>7</sup> and edema.<sup>8</sup> induced by several stimuli like carrageenan, yeast and capsaicin. Variable dose dependent pro and anti-inflammatory effects have been observed with morphine when injected directly into the inflamed site.<sup>9</sup>

Despite extensive investigations on analgesic mechanisms of gabapentin and morphine, not much has been studied about their anti-inflammatory role. The present study was undertaken to investigate the anti-inflammatory effect of gabapentin and

morphine alone and in combination with diclofenac in rat paw edema.

## MATERIALS AND METHODS

Approval for the experimental study was taken from the Institutional Animal Ethical Committee (IAEC) and all the guidelines for the care of animals were followed as per the approved guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) in India. Adult Sprague-Dawley albino rats of either sex, weighing between 150 and 200g, were procured from HAU, Hissar, India and were kept in the institutional animal house for one week before starting the experiments. They had free access to food and water throughout the study.

### Protocol

The healthy adult Sprague-Dawley albino rats (n= 6), weighing between 150 and 200g) of either sex were divided into control and treatment groups. On the day of the experiment the right hind paw of each rat was marked with marker till the level of the lateral malleolus Inflammation induction: Injection 2% v/v formalin, 0.2 ml, was administered subcutaneously on the dorsal surface of right hind paw of the rats to induce inflammation.<sup>10</sup> The paw was immersed in mercury up to this mark. Plethysmograph was used to measure the paw volume at 0, 30, 60, 120 and 240 minutes for the assessment of anti-inflammatory activity. The test groups were compared for change in paw volume with that of untreated control group. Two parameters were recorded: (1) reduction in paw volume (ml) with the help of mercury plethysmometer and (2) percentage inhibition of paw edema which was calculated by applying formula.<sup>11</sup>

% inhibition of edema =  $1 - \frac{\text{Increase in paw vol. in drug treated group}}{\text{Increase in paw volume in saline treated group}} \times 100$ .

#### Drugs and doses

The following drugs were used: Gabapentin, Morphine, Diclofenac and Formalin, all of which were purchased from local market. Gabapentin was dissolved in saline. All drugs were administered intraperitoneally (i.p.) in dosages of 10mg/kg (Diclofenac)<sup>12</sup>, 150gm/kg (gabapentin) and 10mg/kg (morphine)<sup>13</sup> as monotherapy and as combinations of gabapentin+ diclofenac and morphine + diclofenac.

#### Statistical analysis

Paw volume (ml) was determined as (mean  $\pm$  SEM). Statistical analysis was carried out by making use of one-way analysis of variance (ANOVA) to compare different drug treated groups with saline group followed by Dunnet's test,  $p < 0.05$  was considered significant.

## RESULTS

#### Effect of different drugs on formalin-induced rat paw edema: Fig. 1

All drug treatment groups suppressed pedal edema throughout the observation period. Gabapentin, morphine and diclofenac alone as well as combination groups showed a significant ( $p < 0.01$ ) reduction in paw volume at various intervals of time on comparison with saline treated group. The reduction in paw edema with gabapentin and diclofenac was more in comparison to morphine. Combination of gabapentin and diclofenac showed significant inhibition at 240 minutes as compared to individual drugs as well as combination of morphine and diclofenac.

#### Percentage inhibition of paw edema with formalin: Fig. 2

Percentage inhibition of paw volume was more in gabapentin and diclofenac as compared to morphine. Combination of gabapentin and diclofenac showed enhanced reduction in paw edema when compared to gabapentin, morphine and diclofenac alone and combination of morphine + diclofenac groups.

## DISCUSSION

Anti-inflammatory effects of a gamma-aminobutyric acid analogue, gabapentin and opioid agonist, morphine alone and in combination with COX-2 inhibitor, diclofenac were observed against formalin-induced rat paw edema.

Intraplantar injection of formalin in rat induces a localized inflammatory reaction which is a suitable method for the evaluation of anti-inflammatory effects of drugs. When used per se, the drugs gabapentin, morphine and diclofenac, exhibited an anti-inflammatory effect; effect of gabapentin was more compared to that produced by the other two drugs i.e. morphine and diclofenac and it was statistically significant.

The inhibition of paw inflammation by gabapentin, in the present study, is in consonance with another study where in gabapentin demonstrated an anti-inflammatory effect against rat paw edema induced by injecting carrageenan.<sup>14</sup> It has been postulated that, gabapentin affects calcium currents, which might modulate neuronal excitability or release or synthesis of inflammatory mediators thereby alleviating the inflammatory conditions.<sup>10</sup>

Results of morphine, in our study, are comparable to those of another study in which pretreatment with high doses of (7 and 10 mg/kg) intraperitoneally injected morphine showed anti-inflammatory effects in mice.<sup>14</sup> Whereas at low doses i.e. 1mg/kg,

intraperitoneally, it caused increased edema whereas no suppression of edema was observed at moderate doses (3 & 5 mg/kg). It has been postulated that the presence of opioid binding sites on immune cells might be responsible for the anti-inflammatory effects of morphine and to the opioid mediated modulation of several functions of these cells but the mechanism of morphine's immunomodulation is still not completely understood.<sup>14</sup> Some evidences suggest that supraspinal opioid pathways are involved in the immunosuppressive effects of morphine and that these pathways may be distinct from those participating in opioid-induced analgesia.<sup>15</sup>

Treatment with diclofenac significantly enhanced the anti-inflammatory effect and these results are similar to a study in which diclofenac reduced carrageenan induced rat paw edema. Diclofenac, a COX-2 inhibitor, possesses anti-inflammatory effect by inhibiting COX-2 enzyme and thus the synthesis of prostaglandins, leukotrienes, and thromboxanes.<sup>12</sup>

In the present study, simultaneous administration of either gabapentin or morphine with diclofenac caused a significant enhancement of the anti-inflammatory effect as compared to the individual drug and the results are comparable to those of other studies in which combination of both drugs had a greater anti-inflammatory effect as compared to either drug when used alone.<sup>16,17</sup> Similarly, studies involving co-administration of gabapentin with other drugs such as tramadol<sup>18</sup>, morphine.<sup>19</sup> have reported a therapeutic advantage over the individual drug for clinical treatment of pain and inflammation.

## CONCLUSION

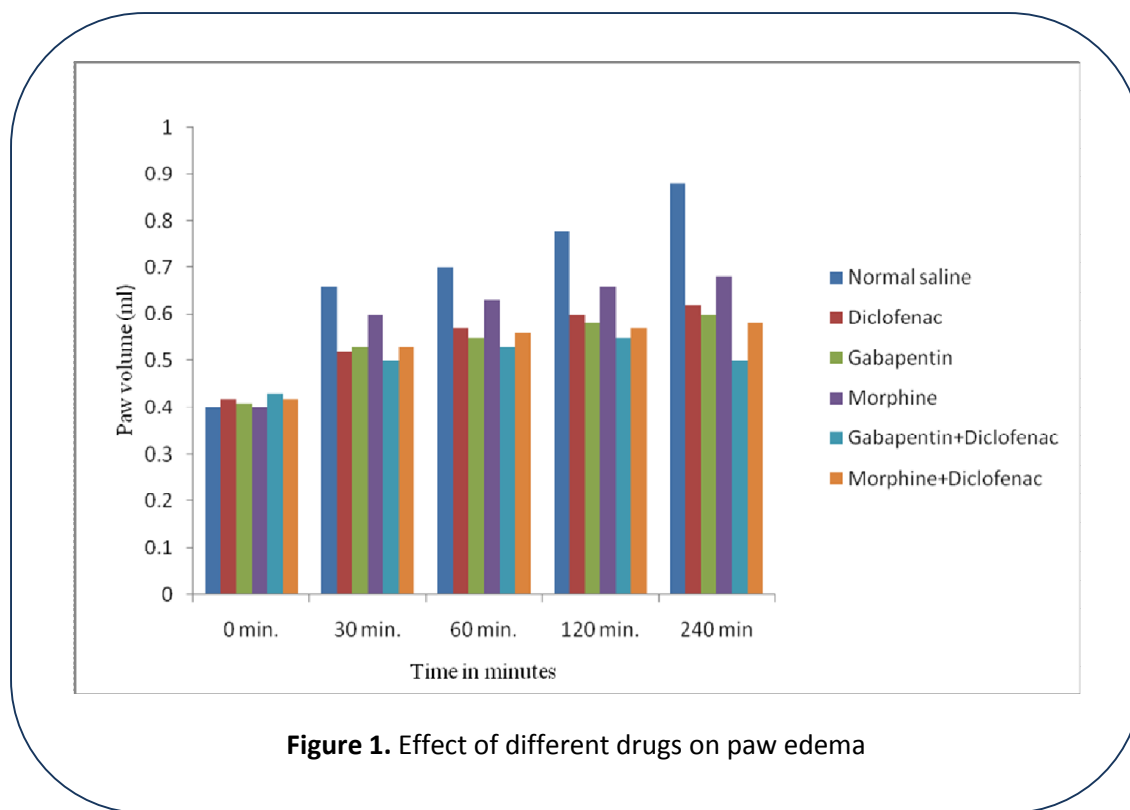
Both gabapentin and morphine have demonstrated anti-inflammatory effects as seen by their response on formalin induced

hind paw edema. However, the mechanisms behind these anti-inflammatory effects are not fully elucidated and need further studies.

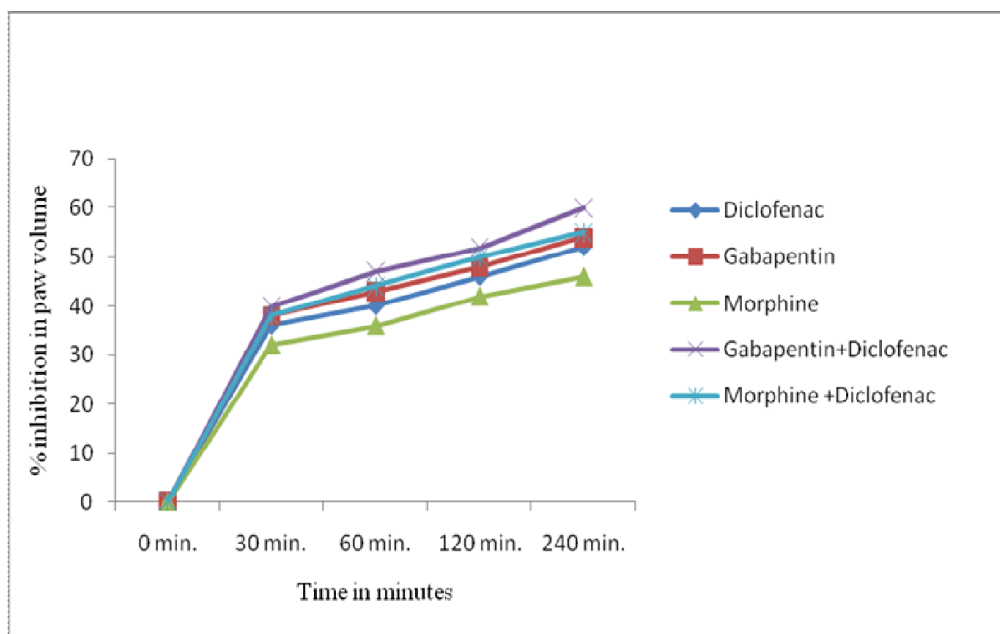
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**Figure 1.** Effect of different drugs on paw edema



**Figure 2.** % inhibition in paw volume in different treatment groups