# EVALUATION OF MODULATORY ROLE OF GABAPENTIN IN INCISIONAL PAIN AND INFLAMMATION.

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**Summery**

Gabapentin, a novel anticonvulsant drug, was found to be an effective anti-nociceptive in several animal models of neuropathic pain. The effect of gabapentin has been studied only in few experimental paradigms of pain, particularly neuropathic pain. The present research work was therefore, focused to further investigate the effect of gabapentin in acute post-operative pain and inflammation.

The effect of gabapentin was evaluated in post-operative pain using incision pain-induced thermal hyperalgesia and cold allodynia in rats and in acute inflammation using acetic-acid-induced vascular permeability in mice. Gabapentin (10, 30 & 100 mg/kg, *po*) was significantly alleviated both thermal hyperalgesia and cold allodynia. Further, Gabapentin (10, 30 & 100 mg/kg, *po*) was shown significant inhibition of acetic acid-induced increased vascular permeability in mice. The results of incisional pain induced hyperalgesia and allodynia are, consistent with the previous reports on the antihyperlagesic effect of gabapentin and shown its effect in combating postoperative pain. The results of anti-inflammatory study revealed the inhibitory effect of gabapentin on vascular permiability. It was suggested that, by possibly binding to the $\alpha_2\delta$ subunit, gabapentin might affect $Ca^{2+}$ currents, which might modulate neurotransmitter release, neuronal excitability or release or synthesis of pain and inflammatory mediators

**Keywords**: Gabapentin; Incisional pain; Hyperalgesia; Allodynia; Nociception; Vascular permeability.

## Introduction

Certain forms of pathological pain are typically resistant to conventional pharmacological treatments (e.g. opioids, nonsteroidal anti-inflammatory drugs). This has resulted in the need to find alternative therapy to manage these types of pains and the recent research have also shown that certain anticonvulsant agents like carbamazepine, oxcarbazepine, lamotrigine, gabapentin, topiramate, felbamate etc. have been used in clinical practice to alleviate certain forms of pain especially lancinating and burning pain, cancer pain etc [1,2,3,4,5]. This indicates that anticonvulsant drugs can be a good option to alleviate above mentioned types pains.

Gabapentin, a novel anticonvulsants drug, was originally developed as a chemical analogue of γ-amino butyric acid (GABA) to reduce the spinal reflex for the treatment of spasticity and was found to have anticonvulsant activity in various seizure models [6]. Gabapentin has attracted recent attention because of its effectiveness against neuropathic pain in both controlled clinical trials and animal models [7].

In particular, gabapentin was effective in several models of neuropathic pain in rats or mice e.g. models of sciatic nerve chronic constriction injury (CCI) [8], spinal nerve ligation [1,8], diabetic neuropathy [6], acute herpes zoster infection [6], thermal injury [9,10] and postoperative pain [6,11]. Also gabapentin was shown to reduce hyperalgesia and inhibit C-fiber responses to noxious stimuli in animal models of inflammatory pain (injection of formalin or carrageenan). The clinical uses of gabapentin in the management of chronic neuropathic pain have been previously reviewed [10]. However there are only a few reports regarding the acute effects of gabapentin on neuropathic pain behaviors in animal model [1,4,5].

The possible mechanisms involved in the multiple therapeutic actions of gabapentin have been actively studied. Several hypotheses were raised. Despite its structural similarity to GABA, gabapentin has no discernible action at $GABA_A$ or $GABA_B$ receptors nor does it have any effect on either the uptake or degradation of GABA. However, it interacts specifically with the $\alpha_2\delta$ subunit of voltage sensitive calcium channels, a subunit ubiquitous to all calcium channel types, suggesting that the $\alpha_2\delta$ subunit is involved in the antinociceptive action of gabapentin [6, 10]. Of the different subtypes, N-type calcium channels acquire greater functional roles after nerve injury and evidence exists for an upregulation of the $\alpha_2\delta$ -1 subunit and the N-type pore-forming $\alpha_1$ or β subunit in this pain state [12]. Pregabalin, a gabapentin analogue, is also effective in the management of neuropathic pain and exerts its pharmacological effects via the same mechanism as that gabapentin. The N-type calcium channel is Cav2.2 and it is unique to sensory nerve terminals in the dorsal horns of the spinal cord controlling neurotransmitter release [6].

By binding to the $\alpha_2\delta$ subunit, gabapentin might affect $Ca^{2+}$ currents to modulate neurotransmitter release or neuronal excitability and synaptic transmission. Gabapentin reduced excitatory amino acid release in the spinal cord in several pain models e.g. 1. Systemic administration of gabapentin decreased the release of glutamate and aspartate in spinal cord, which was elicited by intra-peritoneal injection of acetic acid 2. Gabapentin reduced paw formalin-injection-induced spinal glutamate release in both naive and neuropathic rats [6].

The effect of gabapentin has been studied in several experimental paradigms of pain particularly, neuropathic pain. The present research work was therefore, be focused to further investigate the effect of gabapentin on acute post-operative pain and inflammation in rodents.

## Materials and Methods

***Animals*:**

Healthy albino Wistar rats (150-250 g) and Swiss mice (20-25 g) of either sex procured from Venkateshwara Enterprises, Bangalore, were used. Animals were housed in standard environmental condition in the Institutional animal house and fed with standard pellet rodent diet (Lipton India Ltd., Mumbai). Water was provided *ad libitum*. The experimental protocols were approved by the institutional animal ethical committee of K.L.E.S's College of Pharmacy, Belgaum (Karnataka), India.

***Drugs and regimen*:**

Gabapentin (generously gifted by Dr. Vijay Pal Singh), nimesulide (Nise® tablet); acetic acid, thymol blue and sodium hydroxide (Loba Chemie Pvt. Ltd, Mumbai, India) were used. Gabapentin was dissolved in distilled water. Nimesulide was suspended in 0.5 % carboxy methyl cellulose. All drugs were administered per orally (*po*). Nimesulide (4 mg/kg, *po*) was used as reference standard as it is best effective in both pain and inflammation.

***Analgesic study*:**

**Surgery (Induction of incisional pain in rats):**

Rats were anesthetized by thiopental sodium (25 mg/kg, *ip*) and the plantar surface of the left hind paw was prepared in the sterile manner. A 1 cm longitudinal incision was made with a no. 11 blade, starting 0.5 cm from the proximal edge to the heel extending towards the toes. The plantaris muscle was elevated and incised longitudinally. Following hemostasis with gentle pressure, the skin was apposed with two single nylon sutures. The wound site was covered with povidone-iodine solution and animals allowed to recover in their home cage [13].

**Hyperalgesia and allodynia testing:**

The incised animals were treated with vehicle, nimesulide 4 mg/kg or gabapentin (10, 30 or 100 mg/kg).

Two tests were used to assess the pain behavior: thermal hyperalgesia [14,15] and cold allodynia [13, 14]. Each group contained six (n=6) rats for thermal hyperalgesia test and eight (n=8) rats for cold allodynia test. The tests were performed as described previously with minor modifications. Briefly, animals were tested for pain behavior immediately and at 1, 2, 3, 4 and 5 h after drug administration. The thermal hyperalgesia and cold allodynia was determined by measuring the paw withdrawal latency (PWL) of incised animals and non-incised animals (normal control) by dipping the paw in water bath maintained at 55±0.5°C and 10 ±0.5°C respectively. PWLs of incision control were compared with normal control and PWLs of test groups were compared with incision control.

***Anti-inflammatory study*:**

**Acetic-acid-induced increased vascular permeability in mice:**

Thirty min after the administration of vehicle (saline), nimesulide (4 mg/kg) or gabapentin (10, 30 or 100 mg/kg), each mouse was injected intraperitoneally 0.25 ml of 0.6 % v/v solution of acetic acid. Immediately after acetic acid injection, 10 ml/kg of 5 % thymol blue dye was injected intravenously into the tail vein of the mouse. Thirty min after thymol blue injection, the mouse was sacrificed by cervical dislocation, and the viscera exposed. The viscera were irrigated with 3 ml of saline over a petridish. The exudates was then filtered, and made up to 5 ml. Thereafter, 0.1 ml of 0.1 M NaOH solution was added to exudates to clear the turbidity. The amount of dye leaking out of the capillaries was measured spectrophotometerically at 450 nm ($A_{450}$). Actual concentrations were obtained from a calibration curve plotted with a blank thymol blue. The amount of dye leaking out expressed in micrograms (Mean ±S.E.M.). The amount of dye leaking out represented the vascular permeability index [13].

***Statistical analysis*:**

Data was presented as Mean ± S.E.M. and analyzed for statistical significance using one-way analysis of variance (ANOVA) followed by Dunnet's test. $p<0.05$ was considered to be significant.

## Results

***Hyperalgesia and allodynia*:**

Incision of the skin and fascia plantar surface of the hind paw of rat produced a significant ($p<0.05$) reduction in PWL of incision control as compared to PWL of normal control, indicating incisional pain-induced hyperalgesia and allodynia in rats (Figure1 & 2; table 1 & 2).

The treatment with gabapentin (10, 30 & 100 mg/kg, *po*) showed significant ($p<0.05$) and dose-dependent reversal of the incision-induced thermal hyperalgesia in rats (increased the PWLs) as compared to incision control. The peak effect was observed between 2-4 h after drug administration (Figure1& table 1). The treatment with gabapentin 30 mg/kg at 3 & 5 h and gabapentin 100 mg/kg at 2-5 h showed significant ($p<0.05$) reversal of the incision-induced allodynia in rats as compared to the incision control (Figure 2 & table 2).

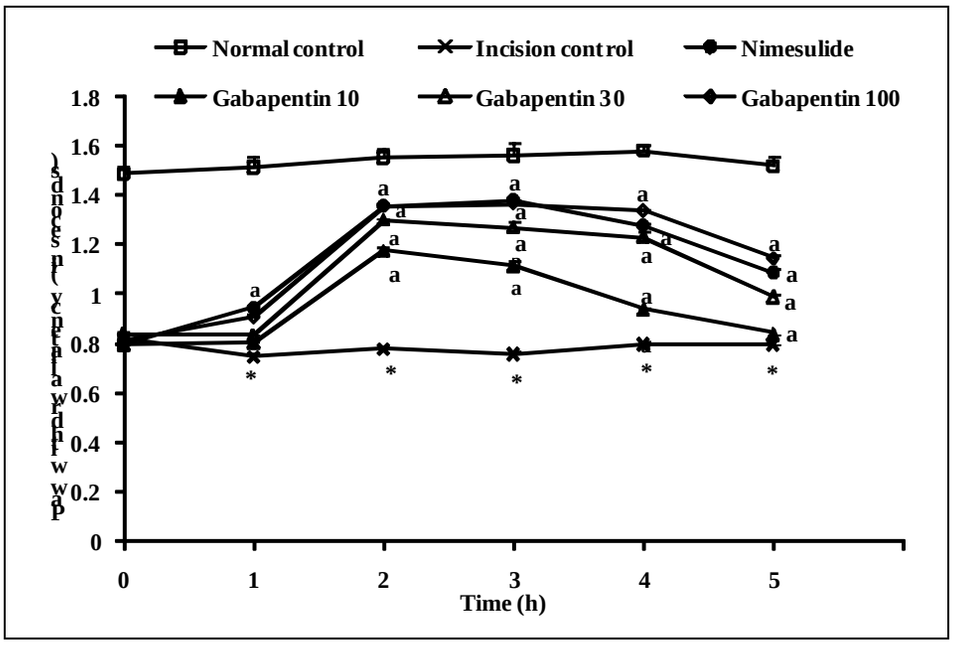
Similarly, nimesulide (4 mg/kg, *po*) showed significant ($p<0.05$) reversal of incision-induced thermal hyperalgesia and cold allodynia in rats as compared to the incision control.

**Table 1: Effect of gabapentin on incisional pain-induced thermal hyperalgesia in rats:**

| Groups | Dose (mg/kg) | PWLs in second | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0 h | 1 h | 2 h | 3 h | 4 h | 5 h |
| **Normal control** | | 1.49 ±0.031 | 1.52 ±0.048 | 1.56 ±0.039 | 1.57 ±0.052 | 1.58 ±0.024 | 1.52 ±0.042 |
| **Incision Control** | **---** | 0.82 ±0.014* | 0.75 ± 0.013* | 0.78 ±0.010* | 0.76 ±0.013* | 0.84 ± 0.005* | 0.80 ±0.005* |
| **Nimesulide** | **4** | 0.80 ±0.008 | 0.95 ±0.011[a] | 1.36 ±0.008[a] | 1.38 ±0.006[a] | 1.28 ± 0.013[a] | 1.09 ±0.023[a] |
| **Gabapentin** | **10** | 0.80 ±0.009 | 0.81 ±0.007 | 1.18 ±0.013[a] | 1.12 ±0.017[a] | 0.94 ± 0.008[a] | 0.84 ±0.005 |
| | **30** | 0.84 ±0.008 | 0.84 ±0.005 | 1.30 ±0.004[a] | 1.27 ±0.026[a] | 1.23 ± 0.027[a] | 0.99 ±0.013[a] |
| | **100** | 0.82 ±0.004 | 0.91 ±0.008[a] | 1.36 ±0.006[a] | 1.37 ±0.006[a] | 1.34 ±0.009[a] | 1.15 ±0.012[a] |

Data expressed as mean±SEM (n = 6). **p<0.05* as compared with normal control; [a]*p<0.05* as compared with incision control.

**Figure 1: Effect of gabapentin (GP) on incisional pain-induced thermal hyperalgesia in rats:**



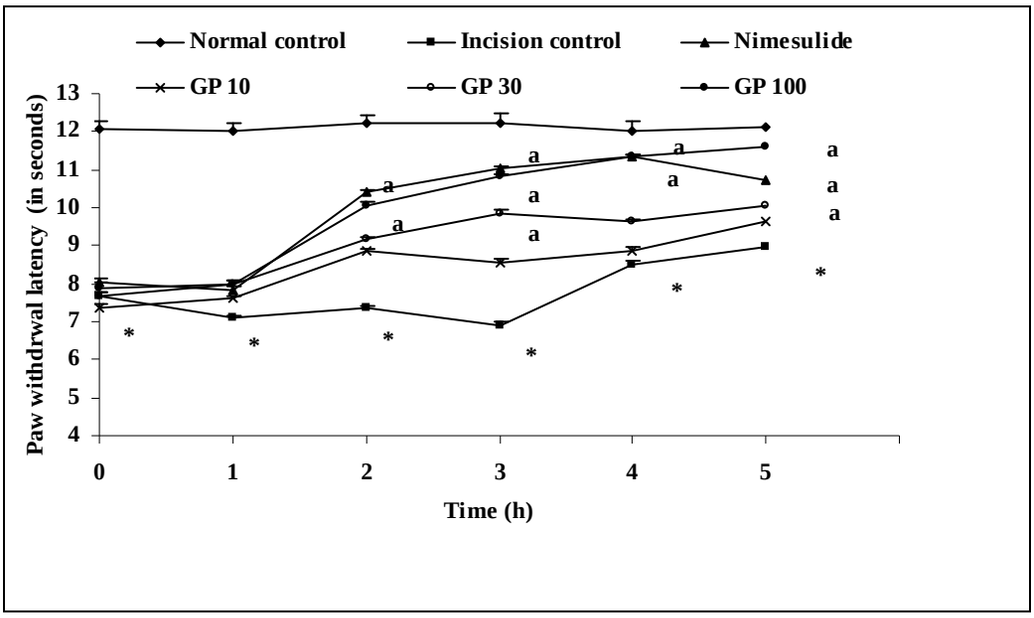

Data expressed as mean±SEM (n = 6). $^{*}p<0.05$ as compared to normal control group; $^{a}p<0.05$ as compared with incision control

**Table 2: Effect of gabapentin on incisional pain-induced cold allodynia in rats:**

| Groups | Dose (mg/kg) | PWLs in seconds | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0 h | 1 h | 2 h | 3 h | 4 h | 5 h |
| **Normal control** | -- | 12.09 ±0.209 | 12.01 ±0.230 | 12.25 ±0.191 | 12.23 ±0.244 | 12.02 ±0.2426 | 12.11 ±0.239 |
| **Incision control** | --- | 7.66 ±0.108* | 7.08 ±0.063* | 7.36 ±0.063* | 6.92 ±0.071* | 8.52 ±0.077* | 8.99 ±0.065* |
| **Nimesulide** | **4** | 8.05 ±0.092 | 7.83 ±0.119 | 10.40 ±0.069a | 11.01 ±0.076a | 11.33 ±0.106a | 10.73 ±0.091a |
| **Gabapentin** | **10** | 7.37 ±0.084 | 7.60 ±0.071 | 8.84 ±0.075 | 8.56 ±0.090 | 8.87 ±0.071 | 9.66 ±0.097 |
| | **30** | 7.66 ±0.108 | 7.96 ±0.100 | 9.16 ±0.043 | 9.85 ±0.090 a | 9.63 ±0.112 | 10.04 ±0.080a |
| | **100** | 7.87 ±0.142 | 8.00 ±0.073 | 10.07 ±0.094a | 10.82 ±0.075a | 11.33 ±0.068a | 11.58 ±0.078a |

Data expressed as mean±SEM (n = 8). *$p<0.05$ as compared with normal control; $^{a}p<0.05$ as compared with incision control.

**Figure 2: Effect of gabapentin (GP) on incisional pain-induced cold allodynia in rats:**




Data expressed as mean±SEM (n =8). $^{*}p<0.05$ as compared with normal control; $^{a}p<0.05$ as compared with incision control.

***Acetic acid-induced increased vascular permeability in mice*:**

The administration of acetic acid (0.6 % v/v, 0.25 ml, *ip*) produced the significant ($p<0.05$) increase in vascular permeability as evident from increased amount of thymol blue leaked in to peritoneal cavity of mice. (Table 3)

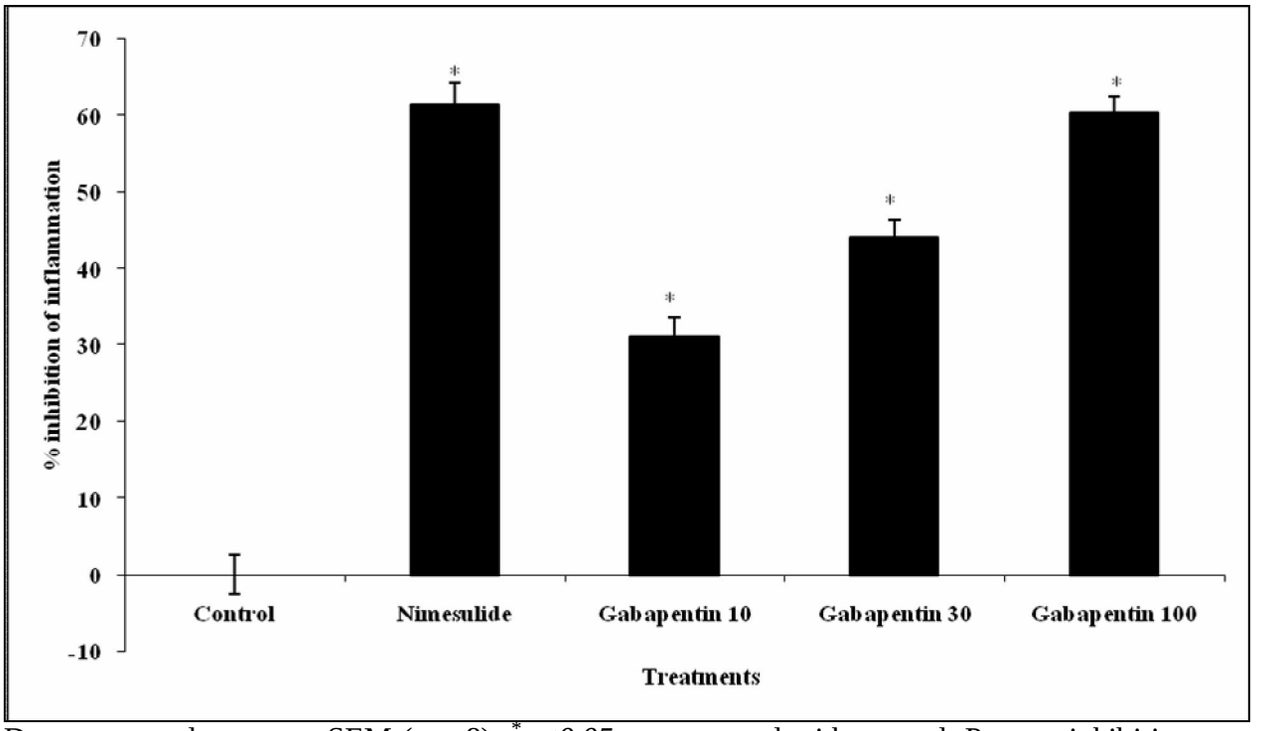
The treatment with gabapentin (10, 30 & 100 mg/kg, *po*) showed significant ($p<0.05$) and dose dependent prevention of (decreased the leakage of thymol blue) the increase in vascular permeability in peritoneal cavity of mice. The percent inhibition of vascular permeability is summarized in table 3 & figure 3.

**Table 3: Effect of gabapentin on acetic acid-induced vascular permeability in mice:**

| Groups | Dose (mg/kg) | $A_{450}$ | Leakage of thymol blue (µgm/mouse) | % Inhibition |
|---|---|---|---|---|
| **Control** | --- | 0.6554±0.0222 | 55.63±2.037 | 0.00±2.560 |
| **Nimesulide** | 4 | 0.2806±0.0188* | 21.38±1.711* | 61.41±2.962* |
| **Gabapentin** | 10 | 0.4616±0.0043* | 21.88±0.383* | 31.18±2.485* |
| | 30 | 0.382±0.0021* | 30.81±0.249* | 44.05±2.191* |
| | 100 | 0.286±0.0093* | 37.94±0.895* | 60.33±2.140* |

Data expressed as mean±SEM (n= 8). *$p<0.05$ as compared with control. Percentages inhibition was calculated with respect to the control (treated with saline).

**Figure 3: Effect of gabapentin on acetic acid induced vascular permeability in mice.**




Data expressed as mean±SEM (n = 8). $^{*}p<0.05$ as compared with control. Percent inhibition was calculated with respect to the control.

## Discussion

One out of every two patients suffers intense pain during the first few days post surgery [16]. Postoperative pain is a common form of acute pain [17] and is a hyperalgesic condition that requires medical attention [13].

Efficacious postoperative analgesia improves patient satisfaction, may decrease morbidity, and perhaps reduce post operative mortality. Pain from a surgical incision occurs at rest and is exacerbated by coughing, ambulation, and mechanical stimulation [17]. Damage to the peripheral nervous system often leads to abnormal pain states referred to as neuropathic pain. The most prominent symptoms include 1. Allodynia – innocuous stimulation evokes abnormally intense, prolonged pain sensations and 2. Hyperalgesia – noxious stimulation evokes abnormally intense and prolonged pain sensations. These sensations can be provoked by both mechanical and thermal (hot or cold) stimulation [7].

Recently, a rat model of postoperative pain was described. Herein, an incision in the skin, and fascia of plantar region of hind paw induced reproducible and quantifiable hyperalgesia lasting several days [11, 17]. The hyperlagesia was quite similar to that seen in human postoperative pain state. An incision of the plantaris muscle of a hind paw induced thermal hyperalgesia and tactile allodynia lasting at least 3 days [11].

Various tests can be used to assess the pain behavior in incision pain model: mechanical hyperalgesia (Randall and Sellitto) [13], thermal hyperalgesia [11], tactile allodynia (von Frey filament) [11] and cold allodynia [13]. The mechanical hyperalgesia, tactile allodynia and decrease in weight bearing were maximal 1-3 days post-incision [16].

The present study examined the effect of gabapentin in incision- induced thermal hyperalgesia and cold allodynia in rat. The treatment with gabapentin (10, 30 & 100 mg/kg, *po*) significantly alleviated thermal hyperalgesia in a dose-dependent manner. The maximal effect was observed between 2 to 4 h post treatments. Whereas the effect on cold allodynia was evident with gabapentin 30 mg/kg at 3 & 5 h and gabapentin 100 mg/kg at 2-5 h post treatments. Earlier studies with gabapentin demonstrated its efficacy to attenuate neuropathic pain behavior in several animal models of peripheral neuropathy. In one such study, gabapentin (30, 100 & 300 mg/kg, *ip*) has been shown to significantly alleviate neuropathy-associated mechanical, thermal and cold allodynia in rats. Similarly, treatment with gabapentin significantly prevented the hyperlagesia symptoms of CCI in rodents [14]. The results of the present study are therefore, consistent with the previous reports on the antihyperlagesic effect of gabapentin and show its effect in combating acute postoperative pain.

The role of pain mediators, primarily prostaglandins derived from cyclooxygenase pathway and leukotrienes derived by lipoxygenase pathway of arachidonic acid metabolism, in post operative pain has been addressed and well understood [13]. Increasing evidence also suggests a potential involvement of ions in modulating postoperative pain and therefore, therapeutic utility of ion channel modulators. In addition, the upregulation of the $\alpha_2\delta$ -1 subunit of calcium channels in pain state is evidenced [6].

Further, present study was aimed to evaluate the anti-inflammatory effect of gabapentin against acetic acid-induced increased vascular permeability in mice.

Mediators of inflammation, such as histamine, prostaglandins and leukotrienes are released following stimulation of mast cells leading to dilation of arterioles and venules. This in turn causes increase in vascular permeability and extravasation of fluid or plasma proteins. The agents that antagonize the effect of histamines, inhibitors of arachidonic acid metabolism and leukotriene receptor antagonists have been reported to reduce the chemogen-induced increase in vascular permeability and limit the exudation sequel. Further, membrane-stabilizing drugs have also demonstrated their ability to oppose the stimuli-induced changes in capillary permeability [18]

In the present study, gabapentin (10, 30 & 100 mg/kg, *po*) significantly and dose-dependently inhibited acetic acid-induced increased vascular permeability in mice suggesting another mechanism by which gabapentin could have shown anti-inflammatory effect in the previous study. It may be presumed therefore, that gabapentin effectively prevents release of inflammatory mediators at the first stage that mounts to anti-inflammatory effect.

It has been evidenced that the calcium movement is an important factor in the activation of cells responsible for inflammation. Calcium does this by releasing the inflammatory mediators or by the activation of the plasma membrane or intracellular enzymes. It has been reported that calcium activates the nitric oxide synthase enzyme, phospholipase $A_2$ and phospholipase C. This results in the activation of the release of arachidonic acid; with resultant formation of prostaglandins, leukotrienes, and thromboxanes. This in turn causes increase in vascular permeability and extravasation of fluid or plasma proteins.[19] It might, therefore, be presumed that the anti-inflammatory activity of gabapentin may be related to the inhibition of the release or synthesis of inflammatory mediators or direct or indirect inhibition of neutrophil infiltration

The results of incisional pain induced hyperalgesia and allodynia are therefore, consistent with the previous reports on the antihyperlagesic effect of gabapentin and shown its effect in combating postoperative pain. The results of anti-inflammatory study revealed that gabapentin was shown inhibitory effect on vascular permeability.

It was, therefore, suggested that, by possibly binding to the $\alpha_2\delta$ subunit, gabapentin might affect $Ca^{2+}$ currents[6], which might modulate neurotransmitter release, neuronal excitability or release or synthesis of pain and inflammatory mediators like prostaglandins, leukotrienes, histamine, 5-hydroxytryptamin, bradykinins or cytokines from sensitized cells.

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