

## Preventive Role of Gabapentin in Transmission of Inflammatory Nociception in Acute and Sub-chronic Inflammatory Models of Rat

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### Abstract

Nociception can trigger an increase in the expression of different genes and their product that can mediate or modulate nociception related changes in the brain. There are a number of experimental model of inflammatory pain provide evidence that nociception results in increased expression of early immediate genes, *c-fos* that are associated with a hyper neuronal activity. It has also been proven that  $\gamma$ -Aminobutyric Acid (GABA), an amino acid neurotransmitter, can involve in inhibitory control of nociception and mediate sensory inputs at the spinal cord level. GABA itself has an effect on the expression of certain genes including *c-fos* in different disease conditions such as seizures. The present study was designed to investigate the effect of Gabapentin, an analogue of GABA, on the expression of *c-fos* in carrageenan induced-acute inflammation. Our results have been shown that in the animal receiving the only carrageenan, there was a marked increase in the expression of the *c-fos* gene in different brain areas with different intensity. Results show that Gabapentin treatment has a potency to inhibit the expression of the *c-fos* gene, which the hallmark of neuronal hyperactivity during inflammatory pain. Gabapentin also prevented the development of other pain-related behaviours, such as paw withdrawal latency responses; we observed that pain scores were not statistically different from baseline. This study suggests that modulating the neurotransmission of nociception can suppress effectively the level of nociception associated with acute inflammation with reducing side effect associated with the extended use of conventional anti-inflammatory drugs.

**Keywords** Nociception; Inflammation; GABA; *c-fos* gene expression; Neuronal hyperactivity inhibition; Animal pain model; Thermal hyperalgesia

### Introduction

Pain is very an unpleasant sensory and emotional state associated with tissue damage (International Association for the Study of Pain (IASP)). Up to 37% of individuals experience chronic pain during their lifetimes and approximately one-fourth of primary care patients with chronic pain also meet criteria for major depression [1]. The stimulus for the pain varies from mechanical to chemical factors. Pain response is the indicator of different underlying pathological condition like inflammation, activated immune systems in infections or tissue injury.

Neuropathic pain developed following nerve injury or any neuronal defects. Following the nerve damage, the gene expression of some involving gene become aggravated including ion channel receptor proteins, which may result in the altered infiltration of some ions especially increased activity of sodium channel with concomitant attenuation of potassium channels at the site of injury. These altered expression status of ion channel proteins on the nerve membrane make neuron hyper-excitably active which lead to ectopic activity, which may the cause of the generation of convulsive pain. The Dorsal Root Ganglia (DRG) present in a dorsal root of a spinal nerve and the cell bodies of greatest proportion of the sensory neurons (afferent neurons) are located within DRG which are responsible for pain maintenance.

Similarly the peripheral nerve injury causing changes in the gene expression which also fasten the activation of various enzyme kinases that promotes different receptor activity including enhanced N-Methyl-D-Aspartate (NMDA) receptor activity and the activation of glial cells especially microglia which in turn release certain chemicals like cytokines (as interleukin-1, tumor necrosis factor alpha), Brain Derived Neurotrophic Factor (BDNF) that exacerbates nociceptive transmission and contributes to the sensitization and maintenance of neuropathic pain. A number of analgesic regimens can be used for pain relief in neuropathic pain, surgery patients, and inflammatory injury. These regimens include the use of sedative anxiolytics, anesthetics including general and local anesthesia [2-4], Non-Steroidal Anti-Inflammatory Drugs (NSAIDs), cyclooxygenase-2 inhibitors and opioids [5-8].

The complex and multiplicity of mechanisms involved in pain requires a multimodal analgesia regimen, furthermore, unfortunately, these available agents have limited efficacy and produce intolerable side effects. Suggesting that a combination of analgesic drugs may improve analgesic efficacy and reduce their associated side effects. Gabapentin [1-(amino methyl) cyclohexane acetic acid], is a structural analogue of  $\gamma$ -Aminobutyric Acid (GABA) is conventionally used as an anti-epileptic agent to treat seizures or convulsions. However, it is also recommended as first line for pain management regime. It has significant effects on diabetic neuropathy, cancer associated pain, neuralgia, inflammation and tissue injury pain [9]. Number of studies suggests that gabapentin inhibit C-fiber and A $\delta$  fiber-evoked responses to noxious stimuli [10]. It is reported that an auxiliary subunit ( $\alpha 2\delta$ -1)

of voltage gated calcium channels is one of the target of gabapentin and by specific binding of gabapentin at  $\alpha 2\delta$ -1 subunits responsible for the pain attenuation action of gabapentin. Studies suggest that the binding of gabapentin at  $\alpha 2\delta$ -1 subunits calcium channels and thus inhibit nerve injury-induced calcium trafficking at plasma membrane of Dorsal Root Ganglion (DRG) neurons and dorsal horn neurons. In addition, It is also shown that gabapentin induce the modulation at its other targets like receptor channels including NMDA receptor, protein kinase C etc. Furthermore, it is reported that gabapentin may also modulate pain or inflammatory regulators i.e. pro-inflammatory cytokines, certain pain regulating genes, in particular, acute phase genes and proteins that do not require any pre-assembled protein machinery i.e., Immediate Early Genes (IEGs), Which contributes to its anti-hypersensitivity action in nociceptive and neuropathic pain. It is believe that any noxious stimulus during pain, can provoke the  $Ca^{2+}$  influx which in turn trigger or enhance the transcription level of immediate early genes within neuronal cells [10,11], Where they regulate cellular phenotype and their function such as adaptation, plasticity, learning, and memory [12-14]. The most important IEG is *c-fos* which is reported to over-express during peripheral and central noxious conditions and can be used as a marker for neuronal activity/excitability [15-17].

The present research work was designed with special focus on the investigating the effect of gabapentin in acute and sub-chronic pain and inflammation using animal model "*carrageenan induced inflammation and hyperalgesia in rats*". To find out, the possible role of gabapentin in acute nociception and its comparison with available analgesic drug which is normally prescribed in routine practice, as these conventional analgesic are highly bounded with severe side effects so, the present study was design in search of better treatment option than conventional treatments.

Since the expression of early immediate gene, *c-fos* gene has been widely used as key marker for neuronal hyperactivity [18,10] during stress conditions including inflammatory nociception, therefore we have used the expression of *c-fos* mRNA as a molecular marker to monitor the neuronal activity in our model of inflammatory nociception and effect of the treatment regime followed in the present study. Furthermore, we also aimed to assess any possible modulatory effect of sub-therapeutic dose of gabapentin when co-administered with sub-therapeutic dose of indomethacin (selective preferential cyclooxygenase-2 inhibitors) against carrageenan induced rat paw edema and neuronal hyperactivity.

## Methods and Materials

### Animals

Female Sprague Dawley (SD) rats weighing 200-250 g (8-10 weeks) were used in this study. The animals were kept at  $21 \pm 2^\circ C$  and on a 12 h light/dark cycle with free access to standard laboratory rat food pellets and water. Rats were randomly distributed to each treatment group. The ethical guidelines of the International Association for the Study of Pain in conscious animals were followed [18] along with the guidelines set by the Scientific Advisory Committee on Animal Care and Use at International Center for Chemical and Biological Sciences, University of Karachi (Protocol No.: 1209004). Rats were randomly distributed to each treatment group of six animals for valid statistical analysis which has 80% power to detect differences in the means.

## Induction and Clinical Assessment of Inflammation

Carrageenan (Sigma Aldrich, Castle Hill, NSW) was dissolved in 0.9% saline to a 1% w/v solution. Paw edema was induced by administered intraplantar injection to left hind paw of mice in a volume of 40  $\mu L$  under anesthesia with a combination of ketamine/xylazine in the dose of 20 g/kg/5 mg/kg (i.p). Behavioral assessment was performed at the time points indicated. For acute inflammatory model, carrageenan was administered once, while for sub-chronic model, 1% w/v solution of carrageenan was administered daily till the end of the experiment i.e., day 4. Clinical severity of inflammation was measured by quantitating the change in the paw volume (as an indicator of edema) with a plethysmometer (model 7140, Ugo Basile, Varese, Italy). The swelling ratio (% swelling) was expresses as the percentage of the increase in paw volume before carrageenan injection. Percent inhibition of the increase in volume which occurs in a control group of rats (vehicle-treated) was determined for each group.

## Drugs and Chemical

Indomethacin, Gabapentin (GBP) and Carrageenan were purchased from Sigma Chemical Company (St Louis, MO, USA). For acute anti-inflammatory and anti-nociceptive evaluation, indomethacin (5 mg/kg), GBP (5 mg/kg) were once administered intraperitoneally. Another separate group received Gabapentin (1.5 mg/kg) in combination with indomethacin (2.5 mg/kg). While, for sub-chronic effects, different animal groups received daily injection of carrageenan (1%), indomethacin (5 mg/kg), Gabapentin (5 mg/kg) and GBP (1.5 mg/kg) with indomethacin (2.5 mg/kg) till the end of the experiment i.e. day 4.

## Thermal hyperalgesia

Nociceptive thermal threshold or thermal hyperalgesia was measured by using a plantar test apparatus, set at  $55^\circ C$  for 25 sec (modified protocol from rats), latency to paw withdrawal and infrared intensity were automatically recorded (Ugo Basile, Italy).

### Reverse transcriptase polymerase chain reaction (RT-PCR) analysis of *c-fos* expression

#### Primer: *c-fos* and GAPDH

As an internal standard (housekeeping gene), GAPDH (glyceraldehyde-3-phosphate dehydrogenase) was used. The primer sequence of the *c-fos* gene and GAPDH gene used in our study is shown in Table 1.

Target gene	Sequences	Annealing temperature ( $^\circ C$ )	PCR product size
<i>c-fos</i> Forward	5'-aacatccccgaaatctac-3'	60	185
<i>c-fos</i> Reverse	5'-agcggacagagaactgga-3'	60	
GAPDH forward	5'-ggaaagctgtggcgtgattgg-3'	60	414
GAPDH reverse	5'-gtagccatgaggtccacca-3'	60	

Table 1: Primer sequences of *c-fos* gene and GAPDH.

#### Tissue Processing and Total RNA extraction

At the end of both experiments i.e. for acute effect and for sub-acute effects, animals were anesthetized with a combination of ketamine/xylazine followed by transcardially perfusion with chilled 0.9% saline. Brain tissue were removed and stored at -80°C for subsequent RNA extraction.

From the brain samples of each group, different brain regions were isolated including amygdala, hippocampus, thalamus, and cortex and subjected to homogenization. To make protein free sample, Proteinase K was added to the homogenate and kept at 55°C for 10 min then homogenized samples were centrifuged at 10,000 rpm for 3 min. The resultant supernatants were processed for total RNA extraction by using RNA easy mini kit, (Qiagen Inc.Valencia) by transferring into RNeasy spin column placed in a collection tube and re-centrifuged for 15 sec at 10,000 rpm. The column membrane was washed twice with the provided buffer at 10,000 rpm for 15 sec. Then, 500 µL buffer RPE was added in the spin column and centrifuged for further 2 min at 10,000 rpm (this step to ensure that no ethanol was remained during RNA elution). RNeasy spin column was placed in a new 1.5-mL collection tube, then 50 µL RNase-free water was added directly to the spin column and centrifuged for 1 min at 10,000 rpm to elute the RNA. The quantification of total RNA was carried out by spectrometric analysis (Abs at 260 nm). An absorbance of 1 U at 260 nm corresponds to 40 µg of RNA per mL i.e., for A260=1, the quantity is 40 µg/mL. The quantification of the total RNA yield was calculated as:

Concentration of RNA sample =  $40 \times A_{260} \times \text{dilution factor}$

Total RNA yield = concentration  $\times$  volume of sample in milliliters

#### cDNA synthesis and RT-PCR

The isolated RNA from each group were reverse transcribed using Superscripts III First-Strand synthesis system (Invitrogen Corporation Carlsbad, CA, USA) for RT-PCR. Briefly, RNA/primer mixture and cDNA synthesis mix were prepared by adding the components provided in the kit in amounts described in the manufacturer's protocol. First, the RNA/primer mixture was incubated at 65°C for 5 min and then re-incubated this mixture after adding cDNA synthesis mix, at 50°C for 50 min after which the reaction was terminated by increasing the temperature at 85°C for 5 min followed by placing the reaction contents on ice for 5 min. The final product i.e., cDNA was stored at -20°C. Negative control was also run and included RT reaction mixture without an enzyme. The transcribed cDNA was then amplified using Omniscript RT kit (Qiagen Inc. Valencia) and primers corresponding to transcripts of the GAPDH and *c-fos*. The PCR mixture for each control reaction was prepared as described in the manufacturer's protocol. To exclude contamination of genomic DNA, reverse transcription was also carried out for the same sample without adding the reverse transcriptase enzyme (negative control). The annealing temperatures of primer for both GAPDH and *c-fos* were optimized at 60°C (Table 1). The mixture containing, products of reverse transcription reactions, primers, and an enzymes were placed in a preheated (94°C) thermal cycler and then denatured for 1 min at 94°C, followed by 30 cycles of amplification in the following manner: *denaturation* at 94°C for 15 sec. then *annealing* at 60°C for 30 sec. and then 1 min *extension step* at 72°C. The final extension step was performed at 72°C for 10 min and upon completion the reactions were hold 4°C.

#### Visualization of PCR Product

The PCR products of each group were electrophoretically resolved on 1% agarose gel containing ethidium bromide. The loading dye

(bromophenol blue) and DNA ladder were used in 1:6 ratios to prepare working DNA ladder. PCR product samples from each group and the DNA ladder were then loaded to the wells of 1% agarose gel and electrophoresed until the bromophenol is near the base of the gel. The bands on the gel were visualized under a UV lamp in a Gel-Dock System (Fluor Chem, Alpha INNOTECH).

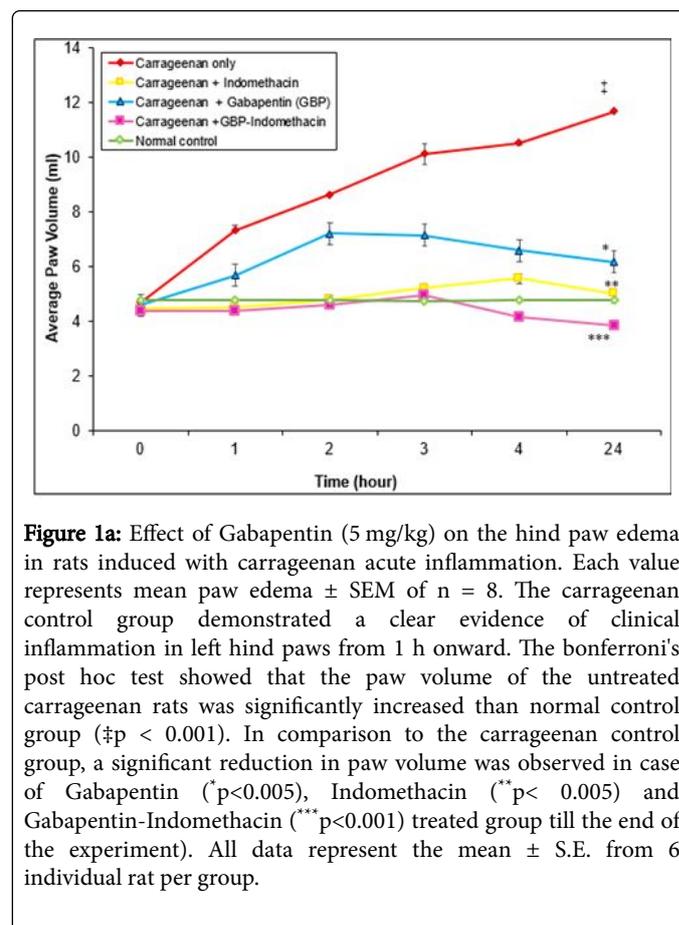
#### Statistical Analysis

To analyze the data, statistical package for the social sciences (SPSS) software was used. Mean  $\pm$  SE of means were used to describe the data in the study. To analyze the RT-PCR images, the image processing program ImageJ (National Institute of Health, USA) was used. The data were analyzed using one-way analysis of variance (ANOVA). The Bonferroni's post hoc test was used to determine which group mean differs. Any values equal or less than the level of 0.05 were considered as significant.

#### Results

##### Clinical Assessment Carrageenan-Induced Inflammation

It was observed that at 1 h post injection of carrageenan, animal group which only received carrageenan, began to show evidence of clinical inflammation in their left hind paw which reached a maximum at 4 h post injection of carrageenan and remained elevated until the last measurement at 24 h post injection as shown in Figure 1a.

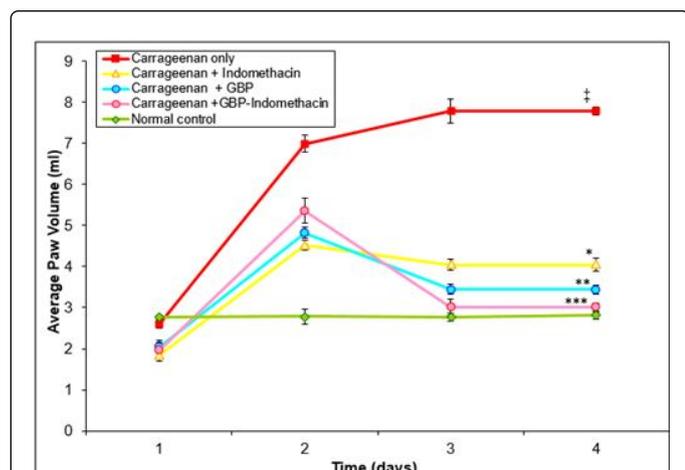


**Figure 1a:** Effect of Gabapentin (5 mg/kg) on the hind paw edema in rats induced with carrageenan acute inflammation. Each value represents mean paw edema  $\pm$  SEM of  $n = 8$ . The carrageenan control group demonstrated a clear evidence of clinical inflammation in left hind paws from 1 h onward. The bonferroni's post hoc test showed that the paw volume of the untreated carrageenan rats was significantly increased than normal control group ( $\#p < 0.001$ ). In comparison to the carrageenan control group, a significant reduction in paw volume was observed in case of Gabapentin ( $*p < 0.005$ ), Indomethacin ( $**p < 0.005$ ) and Gabapentin-Indomethacin ( $***p < 0.001$ ) treated group till the end of the experiment). All data represent the mean  $\pm$  S.E. from 6 individual rat per group.

The clinical inflammation sign which were observed include erythema, swelling around metatarsal joint of left hind paw and increase in paw volume (as an indicator of paw edema). The severity of inflammation was observed significantly aggravated in carrageenan only group ( $p < 0.001$ ) as compared to normal control group, till the end of the experiment. The animal group received Gabapentin (5 mg/kg), showed increase in paw volume at 2 h post carrageenan injection as compared to normal control group but this increase was not as increase as in untreated carrageenan group.

Furthermore, at 3 h post injection of carrageenan, paw volume start to decrease toward baseline and this remain significantly ( $p < 0.005$ ) decreased till the end of the experiment as compared to untreated carrageenan only group. In drug indomethacin (5 mg/kg) treated group, the paw volume was significantly different from untreated carrageenan only group ( $p < 0.005$ ), while further decrease was seen in case of Gabapentin - Indomethacin group ( $p < 0.0001$ ).

The similar pattern was observed in sub-chronic inflammatory pain model as shown in Figure 1b, at day 2 the paw edema was significantly increase in all treated group and also in untreated carrageenan group, later on results shows that there were significantly decrease in paw edema were seen in Gabapentin (5 mg/kg), Indomethacin (5 mg/kg), and in Gabapentin - Indomethacin treated groups till the end of the experiment i.e., day 4 ( $p < 0.0003$ ,  $p < 0.0004$  and  $p < 0.0001$  respectively).



**Figure 1b:** Effect of Gabapentin (5 mg/kg) on the hind paw edema in rats induced with carrageenan sub-chronic inflammation. Each value represents mean paw edema  $\pm$  SEM of  $n=8$ . The carrageenan control group demonstrated a clear evidence of clinical inflammation in left hind paws from 1 h onward. The bonferroni's post hoc test showed that the paw volume of the untreated carrageenan rats was significantly increased than normal control group ( $\#p < 0.001$ ). In comparison to the carrageenan control group, a significant reduction in paw volume was observed in case of Gabapentin ( $*p < 0.004$ ), Indomethacin ( $**p < 0.003$ ) and Gabapentin-Indomethacin ( $***p < 0.001$ ) treated group till the end of the experiment). All data represent the mean  $\pm$  S.E. from 6 individual rats per group.

Percentage (%) inhibition of carrageenan-induced paw edema was calculated for acute effects at 4 h post injection of carrageenan and for sub-chronic effects at day 4. The percentage inhibition of edema was calculated as:

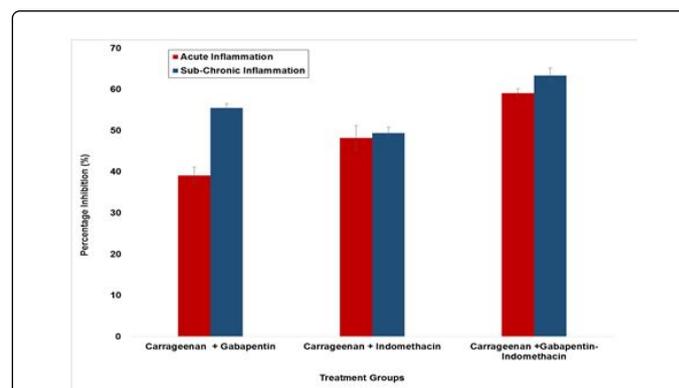
$$\text{Percentage (\% inhibition)} = \frac{\text{paw volume (control group)} - \text{paw volume (treated group)} \times 100}{\text{paw volume (control group)}}$$

In acute experimental study, it was observed that the percentage inhibition value of animal group received Gabapentin (1.5 mg/kg) in combination with indomethacin (2.5 mg/kg) was greater (63.35%) than the percentage inhibition values of all other treated groups. While, the percentage inhibition value of Indomethacin (5 mg/kg) and Gabapentin (5 mg/kg) group were calculated as 49.35% and 39.1% respectively. Similarly, in sub-chronic study, results found that the combination of Gabapentin (1.5 mg/kg) with indomethacin (2.5 mg/kg) was successful in inhibiting the carrageenan-induced paw edema with a percentage inhibition of 59.1%. However, after 4 day treatment with Gabapentin (5 mg/kg) the percentage inhibition became higher (55.5%) than indomethacin (5 mg/kg) treatment (48.2%).

### Effect of Gabapentin on Carrageenan-Induced Hyperalgesia

Our results of carrageenan induced thermal hyperalgesia showed that there was significant increase in paw withdrawal latencies at different experimental time points in treatment groups when compared with the control groups as shown in Figures 2 and 3, Table 2. Gabapentin (5 mg/kg) and indomethacin (5 mg/kg) treated groups showed a greater tolerance of carrageenan-induced thermal hyperalgesia when compared with carrageenan only group.

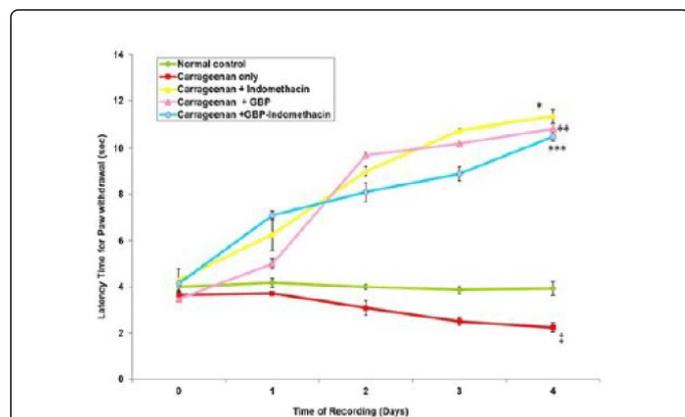
Statistical analysis also revealed that carrageenan-induced hyperalgesia significantly suppressed by Gabapentin (5 mg/kg) and indomethacin (5 mg/kg) ( $p < 0.0001$  and  $p < 0.0002$  respectively) from day 2 till the end of the experiment i.e., day 4. In addition, no significant difference was found in paw withdrawal latency time between the Gabapentin (5 mg/kg), Indomethacin (5mg/kg) and Gabapentin (1.5 mg/kg) in combination with indomethacin (2.5 mg/kg) treated animals. These results indicate that Gabapentin was as effective as indomethacin in reducing inflammation-induced thermal hyperalgesia.



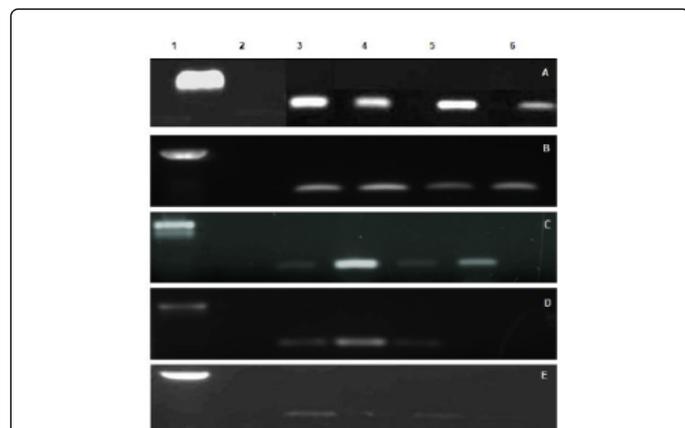
**Figure 2:** Percentage inhibition of carrageenan- induce paw edema in acute inflammation (red) and sub-chronic (blue) treatment of Gabapentin (5 mg/kg), Indomethacin (5 mg/kg) and Gabapentin-Indomethacin.

-	Carrageenam + Gabapentin	Carrageenam + Indomethacin	Carrageenam + Gabapentin - Indomethacin
Acute Inflammation	39.1	48.2	59.1
Sub-Chronic Inflammation	55.5	49.35	63.35

**Table 2:** All data represent the mean ± S.E. from 6 individual rats per group.



**Figure 3:** Time course of paw withdrawal (sec) from the thermal stimulus induced by radiant heat. Repeated administration of Gabapentin (5 mg/kg) induces significant ( $p < 0.001$ ) analgesia against thermal hyperalgesia in rats. All data represent the mean ± S.E. from 6 individual rat per group. Indomethacin alone and in combination with Gabapentin produce similar significant analgesic effects.

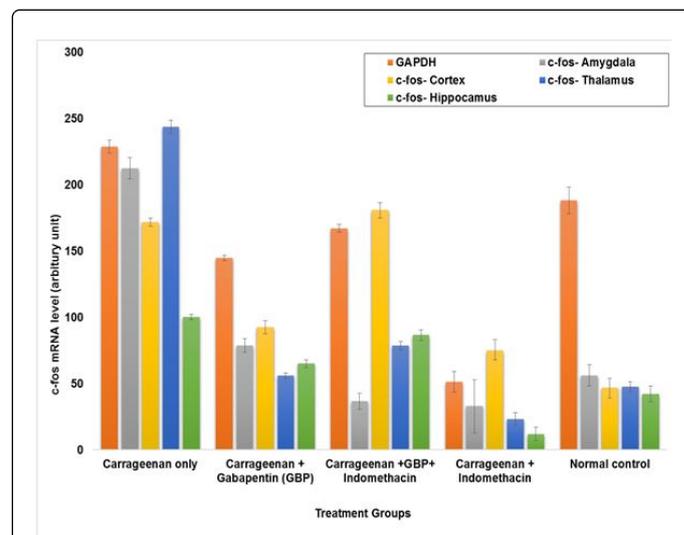


**Figure 4a:** Effects of Gabapentin on *c-fos* mRNA level in acute inflammation. Gel A, B, C, D and E represents carrageenan control, Gabapentin treated group, Gabapentin-Indomethacin, Indomethacin treated and normal controls, respectively. The lanes 1-6 represents GAPDH (414 bp), RT negative, control, and expression of *c-fos* mRNA in amygdala, cortex, thalamus and hippocampus respectively. The *c-fos* levels was normalized to GAPDH mRNA.

### Effect of Gabapentin on *c-fos* gene Expression

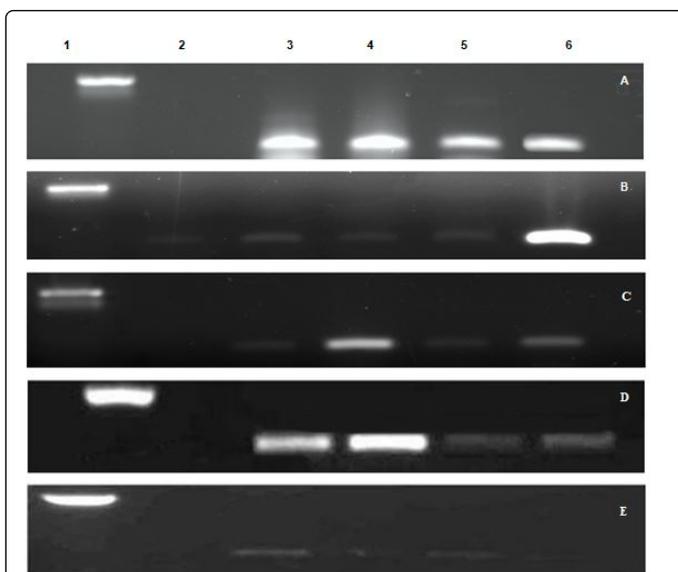
The carrageenan administration caused a marked increase in the *c-fos* gene expression in acute and sub-chronic as shown in Figures 4a, 4b, 5a and 5b. Different brain regions were selected including amygdala, cortex, thalamus and hippocampus to analyze *c-fos* brain expression.

Densitometry analysis revealed that in acute inflammatory pain model *c-fos* is highly expressed in, thalamus, amygdala followed by cortex and finally in hippocampus region. Baseline *c-fos* expression was also observed in normal control group in all four tested regions.

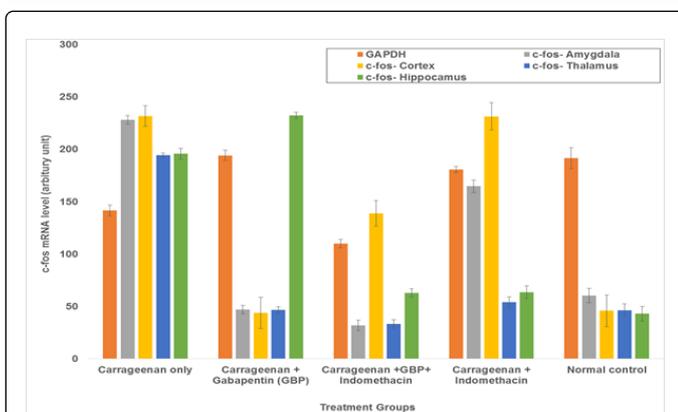


**Figure 4b:** Densitometry analysis of Gabapentin on *c-fos* mRNA levels. The mRNA band on agarose gel were quantified by densitometry and each bar represents the mean SEM.

Within the treatment group, the Gabapentin treatment (5 mg/kg) exhibited significantly reduced *c-fos* mRNA expression in thalamus ( $p < 0.0001$ ), hippocampus ( $p < 0.0001$ ), amygdala ( $p < 0.0001$ ), and then in cortex ( $p < 0.0003$ ) While, indomethacin treatment (5 mg/kg) in carrageenan induce inflammation show significant decrease ( $p < 0.0002$ ) in *c-fos* mRNA expression in hippocampus, thalamus, amygdala and in cortex brain regions. Similarly, Gabapentin treatment (1.5 mg/kg) with indomethacin treatment (2.5 mg/kg) was also successful in reducing significantly ( $p < 0.0002$ ) *c-fos* mRNA level in tested brain regions amygdala, thalamus and hippocampus except cortex brain samples compare to untreated carrageenan induces nociceptive inflammation.



**Figure 5a:** Effects of Gabapentin on *c-fos* mRNA level in sub-chronic inflammation. Gel A, B, C, D and E represents carrageenan control, Gabapentin treated group, Gabapentin-Indomethacin, Indomethacin treated and normal controls, respectively. The lanes 1-6 represents GAPDH (414 bp), RT negative, control, and expression of *c-fos* mRNA in amygdala, cortex, thalamus and hippocampus respectively. The *c-fos* levels was normalized to GAPDH mRNA.



**Figure 5b:** Densitometry analysis of Gabapentin on *c-fos* mRNA levels. The mRNA bands on agarose gel were quantified by densitometry and each bar represents the mean SEM.

## Discussion

It has been reported that in numerous investigations that carrageenan can induce inflammatory hyperalgesia [19-24]. Carrageenan-induced edema in the hind paw was used as a model to determine the progressive relationships between edema formation, expression of certain inflammatory markers both at the site of inflammation induce and in the central nervous system [25-29]. Sensitization of local inflammation lead to the neuronal hyperactivity

in turn, inflammatory nociception trigger the expression of immediate early gene including *c-fos* levels in the brain and can be used as marker for neuronal hyperactivity [30,31]. Available conventional treatment which suppress the nociceptive inflammation have associated with severe peripheral undesirable side effects ranges from ulcer to cardiovascular diseases [31]. Gabapentin which has potent anti-convulsant activity also report to suppress pain threshold with less reported side effects [32,33]. Keeping this in mind, the present study was designed to investigate the preventive effect of Gabapentin on the neuronal hyperactivity during nociceptive inflammation. In this study, any possible inhibitory effect of Gabapentin on *c-fos* gene expression was investigated in carrageenan induced acute and sub-chronic inflammatory pain animal model Also to find is there any synergistic activity of Gabapentin with conventional NSAID indomethacin.

The intensity of pain and inflammation can be assessed by pain specific behavioral studies such as clinical scoring, paw edema and planter induce thermal hyperalgesia [34,35]. Our results evident that in carrageenan untreated group, there was increase in paw edema which is parallel to decrease in paw withdrawal latency in planter hyperalgesia test. Gabapentin and indomethacin treatment suppress the pain threshold which lead in increase paw withdrawal latency times. In addition, no significant difference was found in paw withdrawal latency time between the Gabapentin (5 mg/kg), Indomethacin (5 mg/kg) and Gabapentin (1.5 mg/kg) in combination with indomethacin (2.5 mg/kg) treated animals. These results indicate that Gabapentin was as effective as indomethacin in reducing inflammation-induced thermal hyperalgesia. Interestingly, the percentage inhibition of carrageenan induce inflammation was also significantly increased when Gabapentin was given with indomethacin this indicate convinced synergistic effect between Gabapentin and indomethacin which help in reducing the therapeutic dose of both treatments.

In addition to the clinical scoring and measurement of inflammatory markers, various biomarkers helpful in visualizing the transcriptional changes during nociceptive inflammation. The *c-fos* expression was helpful in providing the insight during nociceptive inflammation and other chronic inflammatory diseases [36-38]. During noxious stimulation, certain brain regions reflect specifically to intensity of neuronal pain and excitability. These areas include amygdaloid nuclei, hippocampus, motor cortex and thalamus brain regions. Our results of RT-PCR confirm these investigating studies, as there were significantly higher mRNA *c-fos* expressions in these brain areas. Our results showed that the Gabapentin (5 mg/kg) and indomethacin (5 mg/kg) treatments in carrageenan induced acute and sub chronic nociceptive inflammation have very potential inhibitory effects as they were not only successful in suppressing the inflammatory symptoms but also prevent the aggravation of *c-fos* mRNA expression levels, i.e., helpful in reducing neuronal excitability during inflammation. Moreover, these two potential treatments i.e. Gabapentin and indomethacin have valuable effects when given synergistically.

## Conclusion

In the present study we found that gabapentin is effective in preventing hyperalgesia induced by carrageenan paw inflammation. It was observed that gabapentin has ability to retain paw edema toward baseline. Our results demonstrate that administration of gabapentin alone and in combination with indomethacin attenuate the carrageenan induce *c-fos* expression, suggested that gabapentin

antagonize the pain probably through a central mechanism of nociception pathways. Combination study with indomethacin suggested that there is potential in combining the drug that is acting through other inflammatory pathways such as cyclooxygenase enzymes, prostaglandins mediators, Ca<sup>2+</sup> ions level in inflammation. This combination therapy control inflammation better with reduce dose of each drug.

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