



Role of Gabapentin as Anti-Inflammatory Agent Alone and Its Modulatory Effect on Co-Administration with Diclofenac Sodium in Rat Paw Edema

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Abstract

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

Methods: After acclimatization of one week, adult winstar albino rats were divided into two groups: control and treatment (n=6). Treatment group received diclofenac (10 mg/kg), gabapentin (20 mg/kg), and gabapentin (20 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 water plethysmometer.

Results: Both 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 with diclofenac groups.

Keywords: Diclofenac, Gabapentin, Water Plethysmometer, Inflammation.

Introduction

Inflammation is the response of living tissue to injury.¹ Inflammation leads to increased vascular permeability; cellular response like chemotaxis of neutrophils; free radical production (H₂O₂, OH),² These inflammatory response are mediated through release of Prostaglandin, cytokines

release (IL-1, TNF-alpha, IL-6, IL-11, IL-8), serotonin, bradykinin, substance P.³ The source of these mediators are the injured tissue itself as well normal tissue, present at the site of injury .

Formalin produces inflammatory pain when injected in hind paw of rat. Formalin induces rapid Ca²⁺ influx via native TRPA-1 channel. Influx of

Ca²⁺ causes release of neurotransmitter at synapse which further augments inflammation. so formalin represent an ideal inflammatory model to investigate anti-inflammatory effect of test compounds.⁴

Gabapentin a structural analogue of GABA and a novel anticonvulsant has been reported to selectively blocks the inflammatory response of the formalin response and carrageenan induced thermal and mechanical hyperalgesia. Both are the animal models of inflammatory pain. The action of gabapentin is mediated through inhibition of the $\alpha 2d$ subunits of voltage-gated Ca²⁺ channels that resemble the Trap-1 channel induced by formalin at nerve terminal.

Despite extensive investigations on analgesic mechanisms of gabapentin, not much has been studied about their anti-inflammatory role as there are only few reports in their supports. The present research work is therefore focused to further investigate and evaluate the effect of gabapentin in inflammatory conditions in rodents by creating inflammatory models using formalin. Further, the present study was aimed to assess any possible modulation of anti-inflammatory effect of gabapentin when co-administered with diclofenac in formalin induced rat paw edema.

Aims and Objectives

1. To study anti-inflammatory activity of drug Gabapentin and compare this effect with diclofenac
2. To Evaluate the Modulatory role of gabapentin on anti-inflammatory effect of Diclofenac when both used in combination and compare this with control group.

Methods

The study was conducted after the approval of IAEC (Institutional Ethical Committee). Adult healthy albino rats of Wistar strain of either sex, weighing between 150- 200gm aged 3-4 months were selected from the Central animal facility. They were kept at a constant temperature of 26±20C and relative humidity of 30-70% under a

12 h dark/light cycle. The animals were fed with standard diet and water *ad libitum*. The rats were acclimatized to the laboratory conditions for seven days prior to test before assigning animals to treatment group. The doses of drugs were based on human daily dose converted to that of rats according to Paget and Barnes (1962).

Drugs and Chemicals: The following drugs were used Gabapentin (Gabapin, 300 mg capsule, INTAS, India), Diclofenac (Voveran D, 50 mg tablets, Novartis, india)

Grouping of animals: Twenty four rats were divided into four groups of six animal each. Group 1 were served as control and received comparable amount of normal saline. group 2 served as standard and received diclofenac (10.0 mg/kg body wt.). Group 3 and 4 received test compound, gabapentin (20.0 mg/kg body wt.) alone and gabapentin with diclofenac in combination respectively.

Anti-inflammatory activity: Thirty minutes after oral feeding of winstar rat with drug, edema was produced in all groups by injecting 0.2 ml of 2% formalin subcutaneously on the dorsal aspect of hind paw of the rats. the paw of each rat was marked with the ink at the level of lateral malleolus and immersed in water of plethysmometer up to this mark. For the assessment of the anti-inflammatory activity, the paw volume was measured plethysmographically at 0 min, 30 min, 60 min, 90 min, 120 min and 240 min. The 0th min reading was considered as the initial paw size of the rats. The change in the paw volume in the test groups was compared with the untreated control groups. two parameters were recorded (1) reduction in paw volume (mL) with the help of water plethysmometer and (2) percentage inhibition of paw edema which was calculated by using the following formulas:

$$\% \text{ inhibition of paw edema} = [(Cf-Ci)-(Tf-Ti)] * 100 / [Cf-Ci]$$

where, at a particular time, Cf = final paw volume of control group; Ci = initial paw volume of control group; (Cf-Ci) = change in paw volume of control group; Tf = final paw volume of test

group; T_i = initial paw volume of test group; $(T_f - T_i)$ = change in paw volume of test group.

Statistical Formulas: Paw volume (mL) was calculated as $(\text{Mean} \pm \text{SEM})$. To compare with different groups with the saline groups, one way analysis of variance (ANOVA) was done followed by t test, $p < 0.05$ was considered significant.

Results and Discussion

The present study was aimed to evaluate the anti-inflammatory effect of Gabapentin in formalin induced edema in rat hind paw using digital plethysmometer. In our study vehicle control group (Formalin induced) showed increase in mean paw volume at regular interval. The displaced volume of water which was 0.71ml at 0 min rose to 1.04 ml at 180 min as shown in Fig.1 showing there is increase in rat paw edema. Intraplantar injection of formalin provokes a local acute inflammatory reaction. It also produced writhing movement in rats which indicates occurrence of pain in rats. Our finding was similar to finding by Goyal et al (2015) that also showed increase in paw volume over time.⁵ The possible explanation could be that formalin causes tissue injury that induces a cascade of cellular reactions in the lesion area, accompanied with the release of pro-inflammatory cytokines, such as $\text{TNF-}\alpha$, $\text{IL-1}\beta$, IL-6 , IL-8 and other substances like bradykinins, serotonin, nitric oxide, which is then followed by subsequent inflammatory reactions.⁶ Prostaglandins such as PGE1 and PGE2 , which are produced at elevated levels in inflamed tissues, increase local blood flow and potentiate the effects of mediators such as bradykinin that induce vascular permeability.⁷ Formalin excites afferent sensory neurons by directly activating TRPA-1 channel that induces rapid Ca^{2+} influx via which formalin activate pain pathway.⁸ In formalin induced inflammation there is increased leukocytes migration as well nociceptive fibers activation leading to pain as suggested by Sir Charles Scott Sherrington.⁹ Our study with Gabapentin revealed that with its use there was initial increase in paw edema value

from 0 to 60 min, value ranging from 0.77 ml to 0.95ml. The mean volume of rat paw start decreasing at 90 min till 180 min ranging from 0.87ml to 0.81ml respectively as shown in table 3. Corresponding percentage inhibition also shows the increase in value from 7 % at 30 min to 35 % at 90 min till 44 % at 180 min. The decrease in paw edema beginning at 90 min and there after continuously decreasing is supported by various test done on gabapentin and various inflammatory model. The findings were similar to study done by Jordana et al (2014).¹⁰ This increase in values was explained on the basis of pharmacokinetics properties of gabapentin which reveals that peak plasma concentration of gabapentin is reached in 2-3 hours i.e. the time when % inhibition was showing an increasing trend.

In our study when compared with vehicle control group the gabapentin showed there is constant increase in the % inhibition and decrease mean paw volume as shown in Fig.4. The possible explanation to this effect can be that gabapentin by binding to $\alpha 2\delta$ subunit of voltage dependent L type calcium channel (VDCC), inhibits release of excitatory neurotransmitters.¹¹ These neuropeptide is known to produces vasodilatation, plasma extravasations, edema, and leukocyte influx, a process termed "neurogenic inflammation."¹² Gabapentin reduces inflammation have been illustrated in various study. Dias et al have found that GBP decreases Myeloperoxidase (MPO), $\text{TNF-}\alpha$, $\text{IL-}\beta$, oxidative stress marker like Malonaldehyde (MDA) and Glutathione (GSH).⁵ Normally there level are increased in inflammation. Camara et al have found GBP increases IL-10 level in tissues, an anti-inflammatory cytokines thus inhibiting flaring of inflammation.¹³ It has been found that during neuropathic inflammation activated macrophages releases IL-2 and more $\text{TNF-}\alpha$. there level was found to decrease in gabapentin pre-treated mice.¹⁴ Gabapentin diminish the release of "pain neuromodulators, fractalkine, Excitatory amino acid like glutamate, substance P, ATP and activation of microglia in the spinal cord by

modulating VDCC $\alpha 2\delta$ -1 subunits, leading to a reduction in thermal hyperalgesia and inflammation.¹⁵

In our study the percent inhibition of rat paw edema after oral administration of diclofenac at 30min, 60min, 90min, 120 min, 180 min were 21%, 22%, 45%, 64%, 74% respectively as shown in Fig.1, 2, 3 and table 1. when compared with % edema at 0 min p-value was found to be <0.01 which was highly significant at all time interval supporting the hypothesis that diclofenac was able to decrease inflammation. These finding were supported by earlier studies by Singh et al (2010), Mulla et al (2010) which have stated 68.2%, 61% inhibition at 180 min.^{16,17} The mean paw volume that changed during this time interval were 0.75 ml to 0.76ml at 0 min and 180 min respectively as shown in table 3 and Fig.4. The possible reason to increased % inhibition could be that the second phase of formalin test is attributed to the release of nociceptive mediators, such as histamine, serotonin, prostaglandin, and bradykinin.^{18,19}

These causes increased production of PG occurs via COX enzyme pathway in the vicinity of injured tissue. Prostaglandins are the major mediators of inflammation. Their inhibition by diclofenac will produce maximum relief from inflammation. When compared with Gabapentin, Diclofenac showed higher % of edema inhibition and more decreased in mean paw volume as shown in fig1-4 and table 1-2 The possible explanation could be that diclofenac cause direct inhibition of PGs synthesis rather than indirect

method as done by gabapentin where inhibiting channels to influence neurotransmitter release and up-regulating IL-10 to inhibit pro-inflammatory cytokines release. Our study also revealed that % inhibition by Diclofenac was maximum at 90, 120, 180 min. This occurred in spite of peak plasma concentration is achieved at 35 min but owing to high binding to plasma protein (99.7%) the maximum concentration at the site of inflammation are achieved after 2-3 hours of peak plasma level.

Diclofenac when combined with Gabapentin the % inhibition of paw edema were found to be superior to either drug when used alone. %inhibition at 30, 60, 90, 120, and at 180 min were 35%, 36.36%, 58%, 69%, 82% respectively. Corresponding mean paw volume also changed from 0.74ml to 0.73 at 0 min and 180 min respectively as shown in table 2. Gabapentin and diclofenac combination groups showed a significant ($p < 0.01$) reduction in paw volume at various intervals of time in comparison to saline treated control group as shown in table 2.

This finding were similar to study done by Goyal et al(2015) who found decrease in mean paw volume and % inhibition of 63% and 76% at 120, 240 min interval.⁵(313). The possible explanation could be combined COX inhibition by Diclofenac and neurotransmitter mediated inflammatory reaction inhibition by Gabapentin at site of tissue injury and inhibiting neuropathic inflammation development thus having greater inhibitory effect on paw edema development in rats.

Observation

Table1: Percentage inhibition of rat paw edema in different treated groups

	0 min	30 min	60 min	90 min	120 min	180 min
Group A	-	-	-	-	-	-
Group B	0	7%	18%	35%	41%	44%
Group C	0	21%	22%	45%	64%	74%
Group B and C	0	35%	36.36%	58%	69%	82%

Table 2. Mean and Standard Deviation with p value of water volume displacement digital plethysmometer

Drugs		0 min	30 min	60 min	90 min	120 min	180 min
Normal Saline	Mean±S.D	0.71 ±0.075	0.85 ±0.049	0.95 ±0.062	0.99 ±0.072	1.02 ±0.065	1.04 ±0.64
	P value		0.001	0.003	0.001	0.000	0.001
Gabapentin	Mean±S.D	0.77 ±0.095	0.90 ±0.079	0.95 ±0.101	0.87 ±0.068	0.83 ±0.67	0.81 ±0.59
	P value		0.003	0.042	0.005	0.014	0.015
Diclofenac	Mean±S.D	0.75 ±0.076	0.86 ±0.80	0.92 ±0.058	0.84 ±0.056	0.79 ±0.58	0.76 ±0.073
	P value		0.001	0.005	0.001	0.016	0.013
Gabapentin +Diclofenac	Mean±S.D	0.74 ±0.077	0.83 ±0.066	0.9 ±0.036	0.81 ±0.047	0.76 ±0.50	0.73 ±0.056
	P value		0.002	0.003	0.006	0.010	0.049

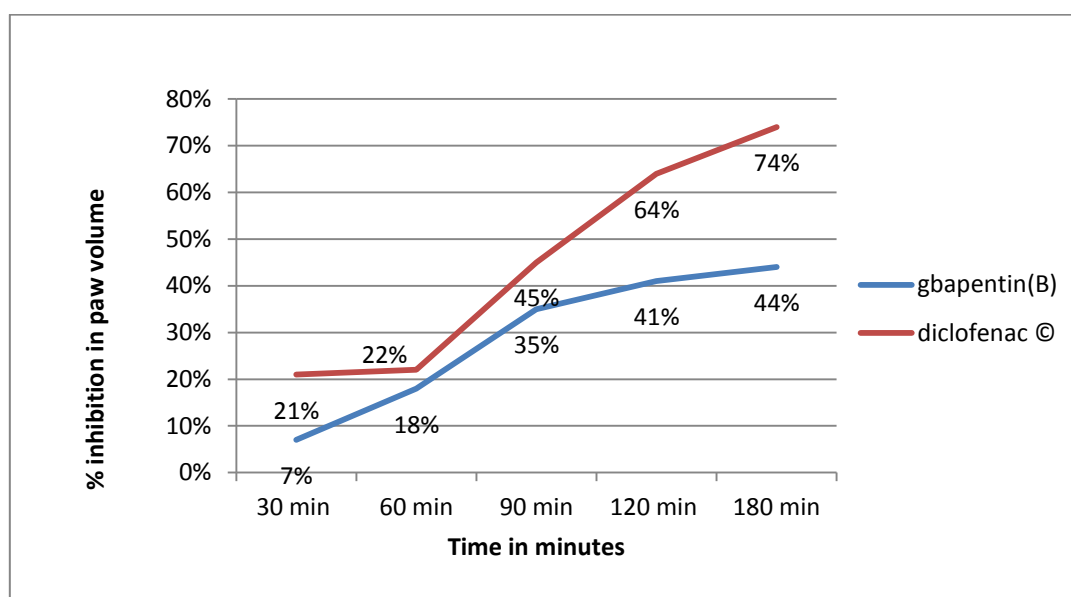


Figure 1: % inhibition in paw volume in different treated groups

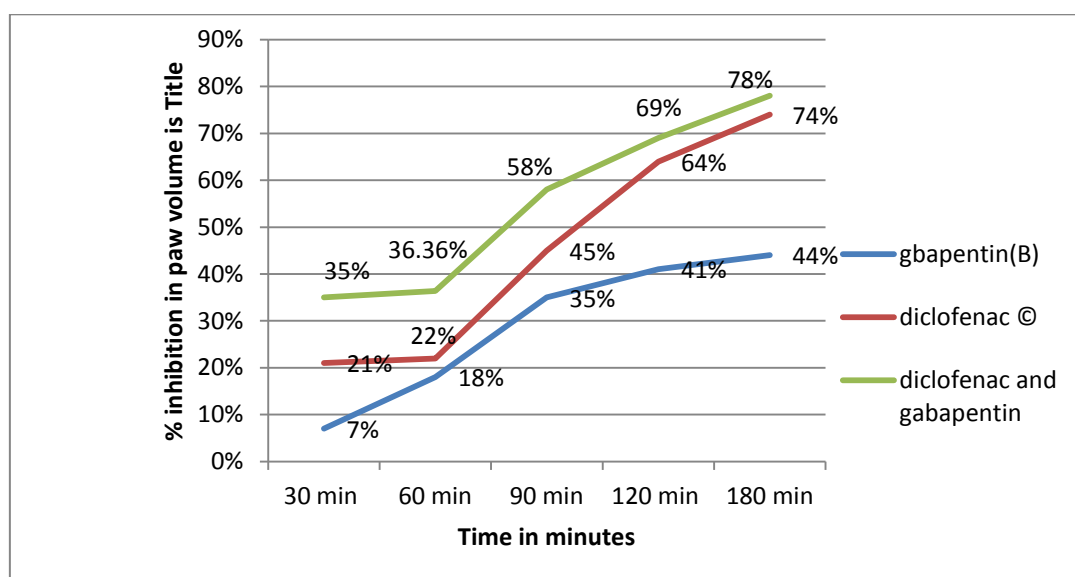


Figure 2: % inhibition in paw volume in different treated groups and their combination

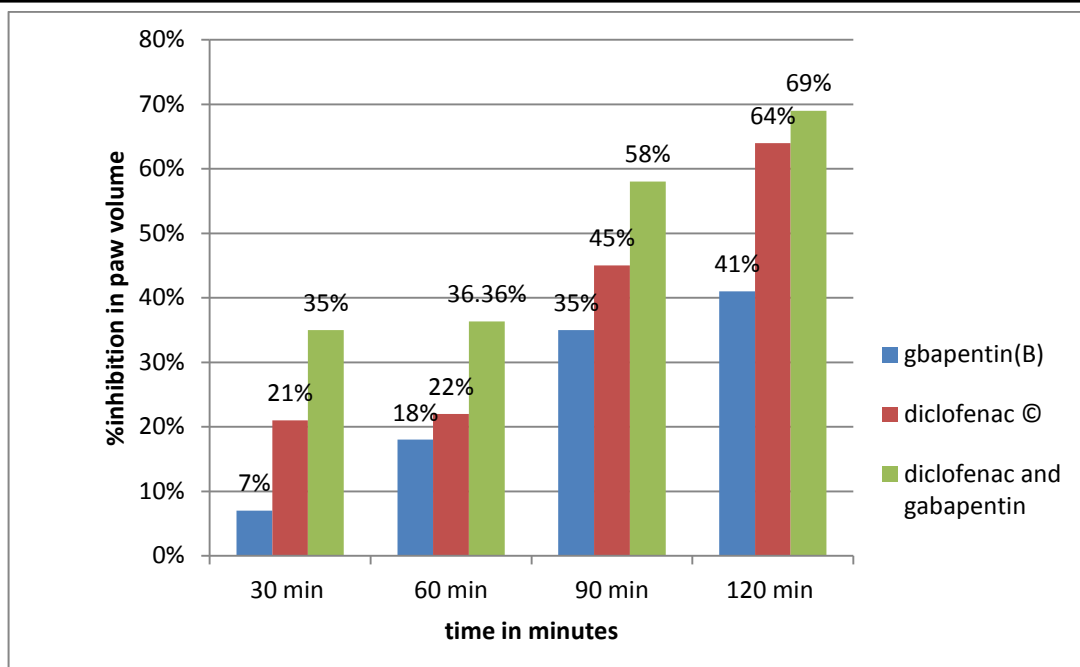


Figure 3: bar diagram showing % inhibition in paw volume in different groups and their combination

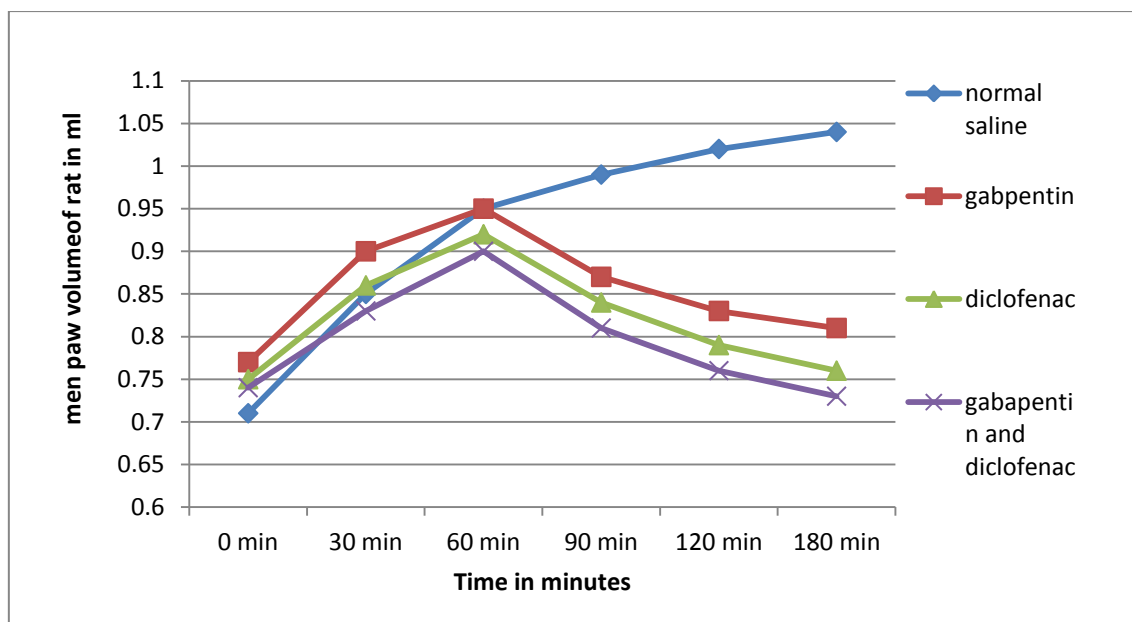


Fig 4 Line diagram showing changes in mean volume of formalin induced rat paw using Normal saline, Gabapentine, Diclofenac and their combination.

Limitation

1. Histo-pathological study of inflamed paw tissue could not be done which would have shown the changing nature of inflammatory process.
2. Only limited drug dose study was done as it was not possible in our settings

Conclusion

From the Finding of our Study, we can conclude that Gabapentin has significant anti inflammatory property in formalin induced paw edema in albino rats. The result is significantly better if it is co-administered with diclofenac as compared, when the drug are used individually.

We can predict that further clinical study of these drugs may give influencing results in patients requiring regular medication for chronic

inflammatory and arthritic pain who are at risk of NSAIDs toxicity.

There is need of big sample size research with more sensitive & specific methods to established gabapentin as a novel anti-inflammatory drug in chronic pain and further human studies are required to establish the same.

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