

## RESEARCH ARTICLE

# Evaluation of analgesic and anti-inflammatory activity of ibuprofen and duloxetine in animal models

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### ABSTRACT

**Background:** Several antidepressants are currently being used as analgesic in neuropathic pain, but their effect on inflammatory pain is not clear. Few studies have shown that duloxetine also has anti-inflammatory effect along with its antinociceptive activity. **Aims and Objectives:** The present study was designed to explore the effect of duloxetine on experimentally induced pain and inflammation in animal models. **Materials and Methods:** The albino Wistar rats of either sex were randomly divided into four groups, i.e., control and ibuprofen, duloxetine (5 mg and 10 mg/kg), with six animals in each group. Analgesia was assessed by hot plate method in rats. Paw edema model in rats after induction with 0.1 mL of 1% carrageenan was used to assess the anti-inflammatory activity. Chronic anti-inflammatory activity was assessed by cotton pellet-induced granuloma. The results obtained were analyzed by analysis of variance followed by Tukey's Honest significance difference *post-hoc* test. **Results:** There was a statistically significant increase in reaction time at all time points compared to control in all treatment groups (ibuprofen and duloxetine) in the hot plate methods. Duloxetine (10 mg/kg) is superior to ibuprofen at every time point as shown by increase in mean reaction time. Duloxetine has also shown significant anti-inflammatory effects compared to control. Ibuprofen was found to cause statistically significant decrease in paw size compared to both control and duloxetine in carrageenan-induced rat paw edema. The anti-inflammatory effect of duloxetine was not significant compared to ibuprofen as seen in cotton pellet-induced granuloma. **Conclusion:** The present study showed analgesic and anti-inflammatory effect of duloxetine in dose-dependent manner. Thus, duloxetine may fit to be an agent offering analgesic and anti-inflammatory along with its antidepressant activity.


**KEY WORDS:** Analgesic; Carrageenan; Inflammation

### INTRODUCTION

Several antidepressants are currently being used as analgesic in neuropathic pain, but their effect on inflammation and inflammatory pain is not clear. Comorbid depression and

chronic pain are highly prevalent. There have been studies to understand the role of chronic inflammation in pain and depression and novel approaches to the development of drugs may emerge that offer improvements in treatment.<sup>[1]</sup> Although the inflammation is a protective phenomenon, sometimes it is uncontrolled and becomes the cause of sufferings by the involvement of surrounding healthy organs and chronic pain.<sup>[2]</sup> In such situations, inflammation needs to be controlled or suppressed by anti-inflammatory mediators to control the disease.

Opioids and nonsteroidal anti-inflammatory drugs (NSAIDs) have been the mainstay of pain treatment for a very long time.

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Over the past 50 years, many new drugs have been introduced for the relief and prevention of pain. In comparison to opioids, NSAIDs are reasonably safe medications when low doses are taken for brief period. NSAIDs such as ibuprofen have been very commonly used and have been found safe to be used as over-the-counter drug.<sup>[3]</sup> However, prolonged use of such drugs may lead to serious side effects and need medical attention.

Hence, newer drugs with different mechanism of action which can be combined with NSAIDs will provide opportunity for effective analgesia at reduced doses. Many analgesic combinations are regularly used in clinical practice.<sup>[4]</sup>

Duloxetine, venlafaxine, and milnacipran belong to the newer class of antidepressant known as serotonin-noradrenalin reuptake inhibitor (SNRI). The agent in this class of drug acts mainly by inhibiting the uptake of noradrenaline and serotonin neurotransmitters and allows more effective control of emotional and physical symptoms of depression.

Duloxetine, one of the new generation serotonin-norepinephrine reuptake inhibitor (SNRI) antidepressants, is used to treat depression and also alleviate allodynia in inflammatory and neuropathic pain. Duloxetine inhibits the reuptake of serotonin and norepinephrine the two important neurotransmitters released from the terminals of descending pain control pathways, thereby increasing their local concentrations and promoting persistence of their analgesic effects.<sup>[5]</sup>

The efficacy of duloxetine in models of persistent pain and neuropathic pain suggests that in addition to its reported antidepressant activity, duloxetine may exhibit efficacy in the treatment of inflammatory pain and other persistent pain conditions in humans.<sup>[6]</sup>

Hence, considering the above findings, the present study was carried out to study the effect of duloxetine on experimentally induced pain and inflammation in animal models.

## MATERIALS AND METHODS

Albino Wistar rats of either sex (100–250 g) were used for analgesic, acute and chronic inflammation study. They were maintained in the animal house for experimental purpose. All the animals were acclimatized for 7 days under standard husbandry conditions. The animals had free access to standard diet with water supplied *ad libitum* under strict hygienic conditions. The study was carried out in the Department of Pharmacology, Government Medical College, Latur, Maharashtra. The approval of the Institutional Animal Ethical Committee was taken before the experiments.

The rats were grouped in separate polypropylene cages and were maintained in a room at ambient temperature of

23±1°C with the help of air coolers and enough humidity on a 12 h light-dark cycle. Similar conditions were provided in laboratory while performing experiments. The study was conducted during daytime (between 10.00 and 18.00 h).

The experimental rats were divided into the following groups each having six animals. All the drugs were given through intraperitoneal (I.P.) route.

- Group (I): Control group (normal saline 10 ml/kg)
- Group (II): Standard group ibuprofen (100 mg/kg)
- Group (III): Duloxetine (5 mg/kg)
- Group (IV): Duloxetine (10 mg/kg).

## Analgesic Method

### Hot plate analgesia

The analgesic activity of test drug was assessed using the hot plate method of Eddy and Leimbach (1953).<sup>[7]</sup> Thirty minutes after dosing group specific drugs, rats were placed on the hot plate. The temperature of the hot plate was maintained at 55 ± 0.20°C.

The time until either licking or jumping (indication of pain) was recorded by a stopwatch. Time interval between placing the animal on hot plate and either licking or jumping was considered as reaction time (latency period).

## Acute Anti-inflammatory Activity

### Carrageenan-induced rat paw edema

The anti-inflammatory properties of the drug were determined by injecting carrageenan in hind paw of each rat as described by Winter *et al.*<sup>[8]</sup> Acute inflammation was produced by injection of carrageenan (0.1 ml of 1% w/v suspension). The paw edema was determined using a plethysmometer modified by Hardayal Singh and Ghosh before and 30, 60, and 120 min after carrageenan injection.

Subcutaneous injection of carrageenan into the rat paw produces inflammation resulting from plasma extravasations, increased tissue water, and plasma protein exudation, along with neutrophil extravasation, due to the metabolism of arachidonic acid.

The first phase begins immediately after injection of carrageenan and diminishes in 2 h, while the second phase begins at the end of the first phase and remains through 3–5 h. Paw is marked with ink at the level of lateral malleolus; basal paw volume is measure plethysmographically by volume displacement method using plethysmometer by immersing the paw till the level of lateral malleolus. The increase in paw volume is calculated as percentage compared with the basal volume. The difference of average values between treated animals and control group is calculated for each time interval and evaluated statistically.

## Chronic Anti-inflammatory Activity

### Cotton pellet-induced granuloma

The method has been described first by Meier *et al.* (1950) who showed that foreign body granulomas were provoked in rats by subcutaneous implantation of pellets of compressed cotton. The amount of newly formed connective tissue can be measured by weighing the dried pellets after removal.<sup>[9]</sup>

Before the start of procedure, sterilized cotton pellets were cut into small circular pellets having weight of  $20 \pm 2$  mg. The cotton pellets were sterilized by autoclaving at  $120^\circ\text{C}$ . The experimental rats were put on fasting for 24 h. Next morning, the experimental rats were anesthetized.

The axilla of each rat was shaved and disinfected with 70% ethanol. An incision was made in the axillary region. By a blunted forceps, subcutaneous tunnels were formed and a sterilized cotton pellet having weight of  $20 \pm 2$  mg was placed on both the axillae.

The surgical wound so formed was suture with catgut. After keeping the animals under observation, the animal was shifted to respective cages and marked accordingly. High level of asepsis and hygienic condition was maintained throughout the experiment. The animals were then dosed accordingly for the next 7 days.

On 8 days of experiment, the animals were anesthetized and the cotton pellets together with the granuloma tissues were carefully removed from all the groups and dried in a hot air oven for overnight at  $60^\circ\text{C}$ . The mean increment in dry weight noted for each group and percentage inhibition calculated.

## Statistical Analysis

All data were presented as mean  $\pm$  SE. Data were evaluated by means of one-way analysis of variance; *post hoc* comparisons were made using Tukey HSD *post hoc* test, to establish the statistical difference between groups. The criterion for statistical significance was fixed at  $P < 0.05$ .

## RESULTS

### Hot Plate Method

Table 1 shows mean increase in reaction time (latency period) on hot plate analgesiometer. There was statistically significant increase in reaction time for all three groups, i.e., ibuprofen, duloxetine (5 mg/kg), and duloxetine (10 mg/kg) compared to control at all time points. Duloxetine (5 mg/kg) is inferior to ibuprofen at 30 min and 60 min, but there was no difference at 2 h and 3 h. Duloxetine (10 mg/kg) is superior to ibuprofen at every time point as shown by increase in mean reaction time.

### Carrageenan-induced Rat Paw Edema

Table 2 shows basal mean paw size and the difference from basal size after giving drugs at 1 h, 2 h, and 3 h. It shows statistically significant decrease in paw size for all three groups, i.e., ibuprofen, duloxetine (5 mg/kg), duloxetine (10 mg/kg) compared to control at all time points. It also shows that ibuprofen is causing statistically significant decrease in paw size compared to both duloxetine 5 mg/kg and 10 mg/kg at all points of time except at 3 h, where the difference between ibuprofen and duloxetine 10 mg/kg is not significant.

**Table 1: Hot plate method**

Drug group	Increase in reaction time (in seconds) Mean $\pm$ SE			
	30 min	60 min	2 h	3 h
Control	0.17 $\pm$ 0.23	1 $\pm$ 0.22	0.83 $\pm$ 0.20	1 $\pm$ 0.19
Ibuprofen	4.66 $\pm$ 0.56 <sup>b</sup>	9.16 $\pm$ 0.98 <sup>b</sup>	8.83 $\pm$ 0.87 <sup>b</sup>	7.66 $\pm$ 0.76 <sup>b</sup>
Duloxetine (5 mg/kg)	2.33 $\pm$ 0.44 <sup>a,c</sup>	5.83 $\pm$ 0.65 <sup>a,c</sup>	8.5 $\pm$ 0.87 <sup>b</sup>	7.83 $\pm$ 0.78 <sup>b</sup>
Duloxetine (10 mg/kg)	5.17 $\pm$ 0.76 <sup>b,c</sup>	17 $\pm$ 1.23 <sup>b,d</sup>	18.5 $\pm$ 1.45 <sup>b,d</sup>	17.17 $\pm$ 1.89 <sup>b,d</sup>

<sup>a</sup> $P \leq 0.05$  versus control, <sup>b</sup> $P \leq 0.01$  versus control, <sup>c</sup> $P \leq 0.05$  versus ibuprofen <sup>d</sup> $P < 0.01$  versus ibuprofen

**Table 2: Carrageenan-induced rat paw edema**

Drug group	Basal (mm) Mean $\pm$ SE	Difference with basal (mm) Mean $\pm$ SE		
		1 h	2 h	3 h
Control	1.58 $\pm$ 0.27	1.91 $\pm$ 0.35	4.91 $\pm$ 0.41	5.41 $\pm$ 0.67
Ibuprofen	1.28 $\pm$ 0.27	0.16 $\pm$ 0.28 <sup>b</sup>	0.21 $\pm$ 0.32 <sup>b</sup>	0.55 $\pm$ 0.24 <sup>b</sup>
Duloxetine (5 mg/kg)	1.55 $\pm$ 0.19	0.78 $\pm$ 0.26 <sup>b,d</sup>	1.55 $\pm$ 0.39 <sup>b,d</sup>	1.12 $\pm$ 0.33 <sup>b,d</sup>
Duloxetine (10 mg/kg)	1.79 $\pm$ 0.10	0.37 $\pm$ 0.45 <sup>b,c</sup>	0.62 $\pm$ 0.44 <sup>b,c</sup>	0.66 $\pm$ 0.38 <sup>b</sup>

<sup>a</sup> $P \leq 0.05$  versus control, <sup>b</sup> $P \leq 0.01$  versus control, <sup>c</sup> $P \leq 0.05$  versus ibuprofen <sup>d</sup> $P < 0.01$  versus ibuprofen

**Table 3:** Cotton pellets induced granuloma

Drug group	Mean weight of cotton wool (mg)		
	After giving drug (Pre-weighted)	After giving drug Mean±SE	% inhibition
Control	20	87.87±2.89	-
Ibuprofen	20	50.50±4.06 <sup>b</sup>	55.06
Duloxetine 5 mg/kg	20	60.38±3.16 <sup>b</sup>	40.6
Duloxetine 10 mg/kg	20	58.71±3.45 <sup>b</sup>	42.96

<sup>a</sup>P<0.05 versus control, <sup>b</sup>P<0.01 versus control, <sup>c</sup>P<0.05 versus ibuprofen <sup>d</sup>P<0.01 versus ibuprofen

### Cotton Pellets Induced Granuloma

Table 3 shows mean increase in weight of cotton wool pellets after giving drugs. The initial weight of cotton pellets was 20 mg. The result shows that there is statistically significant decrease in mean weight for all three groups, i.e., ibuprofen, duloxetine (5 mg/kg), and duloxetine (10 mg/kg) compared to control. There was no significant difference between ibuprofen group as compared to duloxetine group. It also shows that ibuprofen-treated group is showing maximum inhibition of approximately 55% as compared to control group.

### DISCUSSION

This study shows that duloxetine has significant analgesic activity compared to control on hot plate analgesia. It also demonstrated that the analgesic effect increased with increasing dose and duloxetine (10 mg/kg) showed significant analgesic effect compared to ibuprofen. Duloxetine has also shown significant anti-inflammatory effects compared to control. Although ibuprofen was found to have significant anti-inflammatory activity compared to duloxetine in carrageenan-induced rat paw edema, it was found to have comparable anti-inflammatory effect on cotton pellet-induced granuloma.

Pain is perception which is transmitted from the nociceptors to the spinal dorsal horn.<sup>[10]</sup> During this course of pain transmission, it undergoes several modulations. Studies have indicated the role of neurotransmitters in pain modulation, i.e., in peripheral sensitization and in central modulation.<sup>[11]</sup> Duloxetine indirectly increases the level of these neurotransmitters serotonin and norepinephrine. Duloxetine thus can play role in this pain modulation. Various previous studies have been done to demonstrate the analgesic activity of duloxetine. Kuhad *et al.* have found the antinociceptive effect of duloxetine in mouse model of diabetic neuropathic pain, duloxetine significantly and dose dependently increased the nociceptive threshold for thermal hyperalgesia.<sup>[12]</sup> Katsuyama *et al.* demonstrated the antinociceptive effects of duloxetine on vincristine-induced neuropathic pain model in mice. Dose-dependent mechanical allodynia in male ddY strain mice was produced by repeated administration of vincristine (0.05 or 0.1 mg/kg, i.p.). The study demonstrated significant attenuation of vincristine-induced

mechanical allodynia by repeated milnacipran and duloxetine treatment.<sup>[13]</sup> Greish *et al.* studied the effect of duloxetine on vincristine-induced painful neuropathy in rats.<sup>[14]</sup> The finding in our study is consistent to the previous studies though our model was different. Limited study has been done to show anti-inflammatory effect of duloxetine. A study by Choi *et al.* has shown neuroprotective effect of duloxetine due to its anti-inflammatory action.<sup>[15]</sup>

This study evaluated analgesic and anti-inflammatory activity in different models, but the underlying mechanism of the effects was not explored. Due to its different mechanism related to increase in neurotransmitters such as norepinephrine and serotonin which has been implicated in pain pathway, duloxetine and other such drugs present a new approach to pain management. The exact mechanisms, by which antidepressants produce anti-inflammatory effect, are not clear. Multiple studies have shown central mechanisms that modulate peripheral inflammation. The effect of duloxetine on central nervous system to alter neuroimmune interactions could be considered as one of the possible mechanisms of duloxetine-mediated attenuation of inflammation. Further studies are required to confirm these findings in clinical setting.

### CONCLUSION

The results of the present study provide further evidence for the analgesic and anti-inflammatory effect of duloxetine. The clinical implication of the study is that the duloxetine can be used as the analgesic or coanalgesic in patient with depression having pain as comorbidity. Epidemiological data indicate that the chronic depression is associated with the pain as cosymptoms. Similarly, the patients suffering from chronic painful condition also suffer from depression in those patients duloxetine can cater to be an effective analgesic. If a single agent can be effective for such disorder, it can be the most favorable agent. Considering these facts, duloxetine may fit to be an agent offering analgesic, anti-inflammatory activity along with its antidepressant effects.

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