

# DIFERULOYLMETHANE REVERSES CARRAGEENAN INDUCED CHRONIC BILATERAL HYPERALGESIA

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**Abstract :** Although various activities of turmeric have been reported less is known about its role in management of chronic pain and hyperalgesia. Here the ability of turmeric extracts to alter the development of this secondary hyperalgesia induced by carrageenan in rats was assessed. The present study revealed the effect of turmeric extracts and curcumin for its central activities and also contributes to the behavioral response to a diverse range of sensory stimuli. The attenuation of hyperalgesia suggests that diferuloylmethane may be a modulator of muscle derived pain and may suppress the events that lead to the secondary hyperalgesia.

**Key words:-** Turmeric, Hyperalgesia, Carrageenan, Attenuation.

## Introduction

Turmeric has long been used in Ayurveda, Siddha, Unani and Chinese medicine as an anti-inflammatory, to treat digestive disorders and liver problems, and for the treatment of skin diseases and wound healing.<sup>1</sup>The active ingredient in turmeric is curcumin, which has been the subject of numerous animal studies—but as of yet, very few studies on people—demonstrating various medicinal properties.<sup>2</sup> Turmeric powder, curcumin and its derivatives and many other extracts from the rhizome are reported to be bioactive.<sup>3</sup> over the last several years, there has been increasing interest in turmeric and its medicinal properties. This is partially evidenced by the large numbers of scientific studies published on this topic.<sup>4</sup>But less is known about its effect in chronic pain status and its mechanistic behavior in chronic stress condition. While the mechanisms underlying pain derived from musculoskeletal origin remain poorly understand.<sup>5</sup>

Recently animal models have been developed to stimulate muscle derived chronic pain.<sup>6</sup> present study was primarily focused on behavioral comparison of commonly used animal model of chronic pain with chronic pain conditions in humans.<sup>7</sup>

## Materials and Methods

1. **Animals** Wistar rats of either sex (125-160g) were used in the study was maintained at ambient

temperature of 25-30<sup>0</sup>c with food and water ad libitum. Behavioral tests were usually done between 9 a.m. and 2 p.m. All experiments were approved by the institutional ethical committee and were carried out according to the institutional guidelines.

**2. Drugs:** Carrageenan (Sd. fine Chemicals) normal saline (Claris NS NaCL IP 0.9% w/v), Pentazocine, [Curcumin, Ethanolic extract & Petroleum ether extract of *Curcuma longa*] all of which was extracted in your laboratory was used in the current study.

### 3. Induction and assessment of carrageenan-induced inflammatory nociception (hyperalgesia)<sup>6</sup>

#### Induction

Inflammation was induced in the left gastrocnemius muscle belly of rats by injecting 100µl of freshly prepared solution of 3 % carrageenan, in normal saline under light ether anesthesia.

#### Heat testing / assessment<sup>8</sup>

Test animals were tested for behavioral responses to Thermal stimuli by Hot plate method<sup>14</sup>. 6, 10, 12, 14 and 16 days after respective injection test animals were tested for behavioral responses to thermal stimuli of 55<sup>0</sup>c ±2<sup>0</sup>c. They were first placed in glass chambers of a thermal analgesiometer (Space labs, Nashik) and were allowed to acclimate for at least 2 minutes and basal reaction time was taken for each group. Baseline latency to paw withdrawal from thermal source was established thrice, 5 min apart, and averaged. A cut-off time of 15 s was imposed to avoid any injury to the paw. A decrease in

withdrawal latency is interpreted as heat hyperalgesia for the purpose of this study.

### Experimental Protocol

The animals were divided into four groups the following groups (n=6 for each group). First Group was treated with saline (control) and served as negative control. Group2:- Received Pentazocine (5mg/kg) intraperitoneally and served as positive control. Group3:- was treated with curcumin (100 mg/kg) intraperitoneally. Group4:- was treated with Ethanolic extract (100 mg/kg) of *Curcuma longa*. Intraperitoneally. Group5:- was treated with Petroleum ether extract (100 mg/kg) of *Curcuma longa* intraperitoneally.

All the groups (1 to 5) received their respective treatments everyday up to 10th day.

### Statistical analysis

Comparisons were made between test groups using one way ANOVA followed by Tukey-Kramer multiple comparisons test to analyze the data.  $P < 0.01$  were considered statistically significant. All the values were expressed in terms of mean  $\pm$  SEM.

### Results

#### Effect of carrageenan injection in the gastrocnemius muscle

##### A) Mean paw withdrawal latency

The Total Mean basal paw withdrawal latency was calculated in the normal rats/ uninflamed rats for all experimental groups (n = 30) to calculate % antihyperalgesia which was similar approximately at  $4.5334 \pm 0.1105$ .

##### B) Spontaneous pain behavior

The Spontaneous pain behavior signs were observed in animals such as guarding the injected paw and weight bearing on the contralateral paw during the first 24–48 h. After 48 h, there was no sign of spontaneous pain except that there was curling of the paw ipsilaterally still 2 weeks.

##### 2) Effects of Turmeric extracts on heat hyperalgesia

The intraperitoneal administration of **Turmeric extracts** caused a rapid reduction in hyperalgesia returning it to near normal values within 4-5 days in current model of inflammatory muscle hyperalgesia (Tables 1). They avoided the spontaneous pain behavior and an exaggerated response to heat stimuli, a state referred to as hyperalgesia. The effect was maximal after the chronic administration of doses in the drug treated group and effect started sustaining thereafter.

##### % Antihyperalgesia

Maximal inhibition of hyperalgesia was achieved 12, 14 after 16 day after the continual dosing. While on contrary the analgesic effect persisted throughout the study period. The control group showed marked decrease in % antihyperalgesia. While the antihyperalgesic effect of

Turmeric extracts on inflammatory muscle hyperalgesia ranged from (4-77%) approximately. As the Pentazocine treated group showed maximal effect of  $84.0818 \pm 0.2041$  % closely matched by Curcumin  $77.129 \pm 0.1190$  and Ethanolic extract  $54.354 \pm 0.189$  %. The results are shown in shown in Figure no. 1.

### Discussion

The secondary hyperalgesia was induced by intramuscular injection of inflammatory agent carrageenan (3%) in rats. The increased sensitivity is interpreted as a secondary hyperalgesia, because it extends beyond the injected area and includes both limbs. The study demonstrates that curcumin and turmeric extracts can prevent chronic secondary hyperalgesia. As the hyperalgesia did not redevelop after the termination of curcumin and turmeric extracts suggesting that these permanently reversed the hyperalgesia.

The antirheumatic activity of curcumin has also been established in patients who showed significant improvement of symptoms after administration of curcumin<sup>9</sup>. Because of its ability to reduce inflammation, turmeric it helps to relieve the symptoms of osteoarthritis.<sup>10-11</sup> Pain arising from musculoskeletal origin is associated with hyperalgesia, and the model used in this study is proposed to mimic aspects of musculoskeletal pain associated with secondary hyperalgesia.<sup>6</sup> That curcumin stimulates stress induced expression of stress proteins and may act in a way similar to indomethacine and salicylate has also been reported.<sup>12</sup> Curcumin has also been shown to reduce the TNF $\alpha$  induced expression of the tissue factor gene in aortic endothelial cells by representing activation of both AP-1 and NF $\kappa$ B.<sup>13</sup> The anti-inflammatory role of curcumin is also mediated through down regulation of cyclooxygenase-2 and inducible nitric oxide synthetase through suppression of NF $\kappa$ B activation.<sup>14</sup> But NF $\kappa$ B activation does not play any critical role in reversal of the chronic pain conditions.<sup>15</sup>

Recent studies in knock out and transgenic animals have implicated a wide array of ligands and their cognate transduction complexes. [e.g. Acid sensing ion channels, transient receptor potential channels such as TRPV1 (VR1),<sup>16</sup> the voltage gated Na<sup>+</sup> channel, Na<sub>v</sub> 1.8 and ionotropic glutamate receptors] in the central as well as peripheral sensitizing responses that occur in nociceptive pathways after tissue injury as these response contribute to the behavioral correlates of the hyperalgesia in response to a diverse range of sensory stimuli.<sup>17-18</sup>

### Conclusion

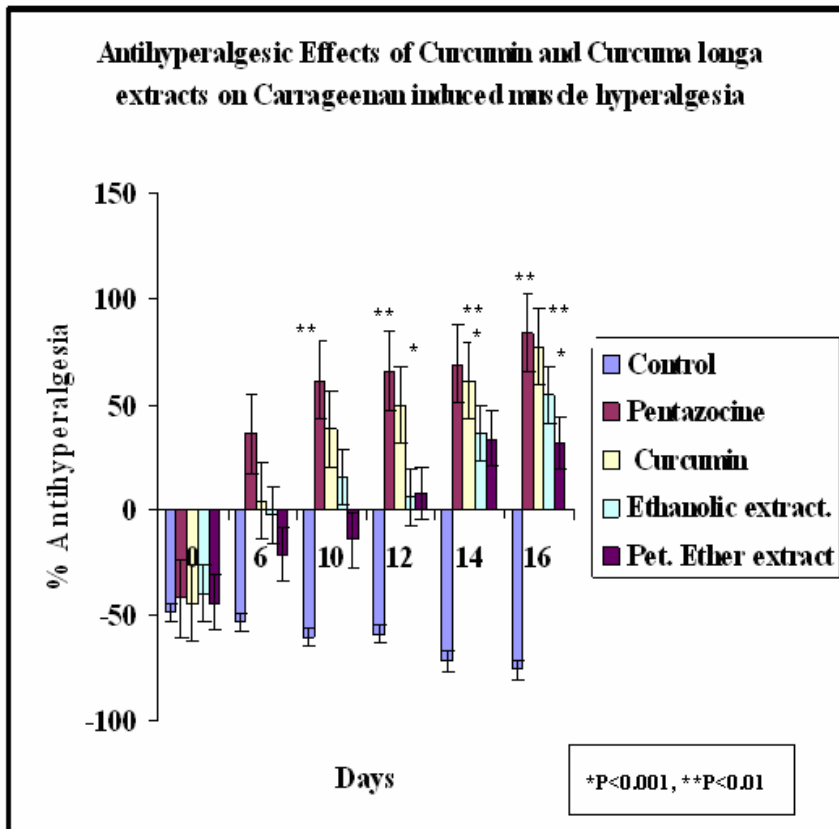
We demonstrated that curcumin can prevent chronic secondary hyperalgesia induced by carrageenan. The attenuation of hyperalgesia suggests that curcumin may be a modulator of muscle-derived pain, and curcumin may suppress events that lead to secondary hyperalgesia triggered by insult to muscle afferents.

Table no. 1:- Effects of Curcumin and *Curcuma longa* extracts on paw withdrawal latency in rats.

Treatments	Paw withdrawal latency in rats in (seconds) after days					
	0	6	10	12	14	16
Control	4.167 ± 0.1667	3.833 ± 0.3073	3.333 ± 0.2108	2.667 ± 0.2108	2.467 ± 0.2108	2.167 ± 0.3073
Pentazocine	4.667 ± 0.333	10.333 ± 0.4216**	12.167 ± 0.3073**	12.50 ± 0.4216**	12.667 ± 0.4216**	13.833 ± 0.3073**
Curcumin	4.50 ± 0.2236	8.0 ± 0.2582	10.500 ± 0.4282*	11.333 ± 0.2667**	11.333 ± 0.4944**	13.333 ± 0.4216**
Ethanolic extract.	4.833 ± 0.3073	6.6167 ± 0.3073	8.843 ± 0.3073*	8.167 ± 0.4773	8.167 ± 0.4773	11.667 ± 0.4216*
Pet. Ether extract	4.50 ± 0.3416	1.049 ± 0.4282	3.667 ± 0.333	8.50 ± 0.7638	8.50 ± 0.8678	10.00 ± 0.5774*

\*P<0.001, \*\*P<0.01 as compared with control, n=6 for each experiment

Figure no 1:



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