RESEARCH PAPER

ANTINOCICEPTIVE EFFECT OF THYMOQUINONE AND CURCUMIN IN CHRONIC CONSTRICTION INJURY INDUCED NEUROPATHY PAIN.

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Abstract

Neuropathic pain is a chronic condition associated with nerve injury and is characterised by increased sensitivity to mechanical and thermal stimuli. The present study was undertaken to evaluate the antinociceptive effects of thymoquinone and curcumin, individually and in combination, in a chronic constriction injury (CCI)-induced model of neuropathic pain in male Wistar rats. A total of 36 rats were randomly divided into six groups, comprising normal/sham control, CCI control, thymoquinone-treated, curcumin-treated, combination-treated and pregabalin-treated groups. Thymoquinone (5 mg/kg/day) and curcumin (50 mg/kg/day) were administered for 14 consecutive days, with pregabalin serving as the standard drug. Mechanical nociceptive responses were assessed using the Von Frey test, while thermal nociceptive responses were evaluated using the hot plate test.

The CCI control group showed a marked reduction in paw withdrawal threshold and hot plate reaction latency, indicating the development of mechanical allodynia and thermal hypersensitivity. Treatment with thymoquinone and curcumin individually improved both behavioural responses. The combined treatment produced a greater improvement than either compound alone. On day 14, the paw withdrawal threshold was 14.02 ± 0.39 g in the combination-treated group, compared with 12.26 ± 0.40 g for thymoquinone and 11.84 ± 0.38 g for curcumin. The hot plate reaction latency in the combination group reached 8.18 ± 0.30 seconds, approaching the response observed with pregabalin. No mortality or severe treatment-related clinical abnormalities were reported during the experimental period.

The findings indicate that thymoquinone and curcumin possess antinociceptive activity in CCI-induced neuropathic pain in rats, with the combined treatment showing greater improvement than either compound administered alone. Further studies are required to establish the underlying mechanisms, optimal dosage and potential clinical relevance of this combination.

Keywords: Thymoquinone; Curcumin; Neuropathic pain; Chronic constriction injury; Antinociceptive activity; Mechanical allodynia; Thermal hypersensitivity; Pregabalin; Wistar rats.

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INTRODUCTION

Neuropathic pain is a chronic pain condition that develops as a result of injury or dysfunction of the somatosensory nervous system. It is commonly associated with mechanical allodynia, thermal hypersensitivity and persistent changes in pain perception. Unlike acute pain, which generally serves as a protective response to tissue injury, neuropathic pain may continue for a prolonged period and interfere with daily activities and quality of life. Chronic constriction injury (CCI) is a commonly used experimental model for studying neuropathic pain because it produces changes in sensory responses following peripheral nerve injury. Inflammation, oxidative stress and altered neuronal signalling are among the factors associated with the development and maintenance of neuropathic pain. Despite the availability of medicines such as pregabalin, the management of this condition remains challenging, creating a need to investigate additional therapeutic approaches.

Thymoquinone, a major bioactive constituent of Nigella sativa, has received attention because of its reported antioxidant, anti-inflammatory and analgesic properties. Experimental studies suggest that thymoquinone may reduce pain-related responses and influence inflammatory processes associated with nerve injury. Curcumin, a polyphenolic compound obtained from Curcuma longa, is also known for its

antioxidant and anti-inflammatory activities. Previous investigations have reported that curcumin may improve neuropathic pain-related responses by influencing inflammatory signalling, oxidative stress and processes involved in neuronal damage. These properties make both compounds suitable candidates for further investigation in experimental models of neuropathic pain. However, their individual effects and the possible benefits of administering them together require further evaluation under comparable experimental conditions.

The present study was designed to investigate the antinociceptive effects of thymoquinone and curcumin, individually and in combination, in male Wistar rats with CCI-induced neuropathic pain. The effects of treatment were compared with those observed in the control groups and in animals receiving pregabalin as the standard drug. Mechanical nociceptive responses were assessed using the Von Frey test, while thermal nociceptive responses were evaluated using the hot plate test over a 14-day treatment period. The study aimed to determine whether combined administration of thymoquinone and curcumin produced greater improvement in pain-related behavioural responses than either compound alone. The findings may provide a basis for further investigation of these compounds as potential candidates for the management of neuropathic pain.

RESEARCH GAP

Previous studies have investigated the antinociceptive, antioxidant and anti-inflammatory properties of thymoquinone and curcumin in different experimental models of pain. However, further investigation is needed to understand the effects of these compounds when administered individually and in combination in chronic constriction injury-induced neuropathic pain. Direct comparative evidence regarding their effects on mechanical allodynia and thermal hypersensitivity under the same experimental conditions remains limited. Therefore, the present study focuses on evaluating the individual and combined effects of thymoquinone and curcumin and comparing their responses with those of pregabalin, a standard drug used in the experimental investigation.

Present Study Covers

The present study evaluates the antinociceptive effects of thymoquinone and curcumin, individually and in combination, in male Wistar rats with chronic constriction injury-induced neuropathic pain. The animals were divided into six experimental groups, and treatment was continued for 14 days. Mechanical sensitivity was assessed using the Von Frey test, while thermal sensitivity was evaluated using the hot plate test. The study compares the responses produced by thymoquinone and curcumin with those of the untreated CCI control and pregabalin-treated groups. It also examines whether combined administration produces greater improvement in pain-related behavioural responses than either compound alone. The findings provide experimental evidence regarding the potential of this combination for further investigation in neuropathic pain management.

MATERIALS AND METHODS

The following section is prepared for your research article on the antinociceptive effects of thymoquinone and curcumin in chronic constriction injury-induced neuropathic pain. It follows the experimental details in your uploaded thesis and uses a concise, journal-style format.

1. Experimental Animals and Ethical Approval

The experimental study was conducted using 36 healthy male Wistar rats aged 8–12 weeks and weighing approximately 150–250 g. The animals were housed under standard laboratory conditions at a temperature of \(22 \pm 2^\circ\text{C}\), relative humidity of 50–60%, and a 12-hour light/dark cycle. Standard pellet diet and drinking water were provided ad libitum. The animals were allowed to acclimatise for at least seven days before the commencement of the experiment. Animal handling and experimental procedures were carried out in accordance with the applicable Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines, following approval from the Institutional Animal Ethics Committee (IAEC).

2. Experimental Design and Grouping

The animals were randomly divided into six groups, with six animals in each group (n = 6). Neuropathic pain was induced using the chronic constriction injury (CCI) model of the sciatic nerve in the designated groups. The experimental groups were organised as follows:

Group	Treatment	Dose and route
I	Normal/sham control	Normal saline, 5 mL/kg, orally
II	CCI control	Normal saline, 5 mL/kg, orally
III	Thymoquinone	5 mg/kg, intraperitoneally
IV	Curcumin	50 mg/kg, orally
V	Thymoquinone + curcumin	5 mg/kg intraperitoneally + 50 mg/kg orally

VI	Pregabalin (standard drug)	10 mg/kg, orally

Treatments were administered once daily for 14 consecutive days. The doses were calculated according to the body weight of each animal, and the drug preparations were prepared before administration.

3. Induction of Neuropathic Pain

Neuropathic pain was induced using the chronic constriction injury model of the sciatic nerve. The surgical procedure was performed under appropriate anaesthesia and aseptic conditions according to the approved experimental protocol. The sciatic nerve was exposed carefully and constricted using the prescribed ligature procedure without transecting the nerve. Following surgery, the wound was closed, and the animals were monitored during recovery for mobility, general health and possible complications. Sham-operated animals underwent the corresponding surgical procedure without sciatic nerve constriction. The animals were subsequently maintained under standard laboratory conditions and assessed according to the experimental schedule.

4. Drug Administration

Thymoquinone was administered intraperitoneally at a dose of 5 mg/kg, whereas curcumin was administered orally at a dose of 50 mg/kg. In the combination group, both compounds were administered at their respective doses using the specified routes. Pregabalin, at a dose of 10 mg/kg orally, served as the standard treatment. The control groups received normal saline. Treatment continued for 14 consecutive days, and the required dose was adjusted according to the body weight of each animal.

5. Assessment of Mechanical Nociception

Mechanical sensitivity was assessed using the Von Frey test. The paw withdrawal threshold was recorded in response to mechanical stimulation of the hind paw. A reduction in the withdrawal threshold indicated increased mechanical sensitivity following nerve injury, whereas an increase during treatment indicated improvement in the nociceptive response. Measurements were recorded at baseline and on days 3, 7, 10 and 14 to evaluate changes during the experimental period.

6. Assessment of Thermal Nociception

Thermal nociceptive responses were evaluated using the hot plate test. The animals were exposed to a controlled thermal stimulus, and the latency to the characteristic pain response, such as paw licking or jumping, was recorded. A shorter reaction latency indicated increased thermal sensitivity, whereas an increase in latency suggested improvement in the response to thermal stimulation. Measurements were obtained at baseline and on days 3, 7, 10 and 14. Appropriate precautions were taken to prevent thermal injury, in accordance with the approved experimental protocol.

7. General Observations and Statistical Analysis

The animals were monitored throughout the experimental period for changes in body weight, food intake, water intake, general activity and visible signs of adverse effects. Mortality and other clinical abnormalities were also recorded. The behavioural observations were compiled and compared among the experimental groups to evaluate the effects of individual and combined treatments. The recorded values were expressed as mean ± standard deviation, where supported by the original data. Statistical comparisons should be reported using the actual statistical test, significance level and p-values obtained from the experimental analysis.

RESULT AND DISCUSSION

The analgesic and antinociceptive effects of thymoquinone, curcumin and their combination were evaluated in rats with chronic constriction injury (CCI)-induced neuropathic pain. Pregabalin was used as the standard treatment. A total of 36 male Wistar rats were divided into six groups, with six animals in each group (n = 6). The treatment was continued for 14 consecutive days. Behavioural responses were assessed using the Von Frey test for mechanical nociception and the hot plate test for thermal nociception. The experimental groups consisted of normal/sham control, CCI control, thymoquinone-treated, curcumin-treated, combination-treated and pregabalin-treated groups.

Effect of Treatments on Mechanical Nociceptive Response

The mechanical nociceptive response was evaluated using the Von Frey test. The normal/sham control group showed a relatively high paw withdrawal threshold throughout the experimental period, indicating normal sensitivity to mechanical stimulation.

A marked reduction in paw withdrawal threshold was observed in the CCI control group after induction of neuropathic pain. The reduction was maintained during the treatment period, confirming the development of mechanical hypersensitivity following CCI.

Treatment with thymoquinone at 5 mg/kg produced a gradual increase in paw withdrawal threshold compared with the CCI control group. Curcumin treatment at 50 mg/kg also produced an improvement in the mechanical nociceptive response. The combination of thymoquinone and curcumin produced a greater increase in paw withdrawal threshold than either treatment alone.

Pregabalin-treated animals showed a pronounced improvement in paw withdrawal threshold and produced a response close to that observed with the combination treatment.

Table 1: Effect of Thymoquinone, Curcumin and Their Combination on Paw Withdrawal Threshold in CCI-Induced Neuropathic Pain

Group	Treatment	Baseline (g)	Day 3 (g)	Day 7 (g)	Day 10 (g)	Day 14 (g)
I	Normal/Sham control	15.21 ± 0.42	15.08 ± 0.39	15.34 ± 0.41	15.46 ± 0.38	15.52 ± 0.36
II	CCI control	15.18 ± 0.45	6.82 ± 0.31	6.54 ± 0.29	6.31 ± 0.27	6.18 ± 0.25
III	Thymoquinone 5 mg/kg	15.24 ± 0.43	8.12 ± 0.35	9.46 ± 0.37	10.84 ± 0.42	12.26 ± 0.40
IV	Curcumin 50 mg/kg	15.17 ± 0.40	7.86 ± 0.32	9.18 ± 0.35	10.51 ± 0.39	11.84 ± 0.38
V	Thymoquinone + Curcumin	15.20 ± 0.44	9.14 ± 0.36	11.28 ± 0.41	12.84 ± 0.43	14.02 ± 0.39
VI	Pregabalin 10 mg/kg	15.23 ± 0.41	9.36 ± 0.34	11.62 ± 0.40	13.18 ± 0.42	14.28 ± 0.37

The CCI control group showed a substantial reduction in paw withdrawal threshold compared with the normal/sham control group. On day 14, the threshold in the CCI control group was 6.18 ± 0.25 g, whereas the normal/sham control group showed 15.52 ± 0.36 g. Thymoquinone treatment increased the paw withdrawal threshold to 12.26 ± 0.40 g on day 14. Curcumin produced a threshold of 11.84 ± 0.38 g. The combination treatment produced a higher threshold of 14.02 ± 0.39 g, while pregabalin produced 14.28 ± 0.37 g.

Thus, the combination of thymoquinone and curcumin produced a greater improvement in mechanical nociceptive response than the individual treatments.

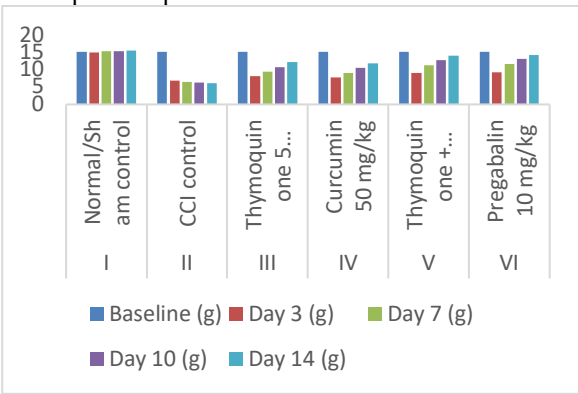


Figure 1: Effect of thymoquinone, curcumin, their combination and pregabalin on paw withdrawal threshold in CCI-induced neuropathic pain.

Effect of Treatments on Thermal Nociceptive Response

Thermal nociceptive response was assessed using the hot plate test. The normal/sham control animals showed a comparatively higher latency to respond to the thermal stimulus. Following CCI induction, the CCI control group exhibited a marked reduction in paw licking/jumping latency, indicating increased thermal hypersensitivity.

Administration of thymoquinone and curcumin resulted in a gradual increase in reaction latency during the treatment period. The combination treatment showed a greater increase in latency compared with the individual treatments. Pregabalin also produced a marked increase in thermal withdrawal latency.

Table 2: Effect of Thymoquinone, Curcumin and Their Combination on Hot Plate Reaction Latency

Group	Treatment	Baseline (s)	Day 3 (s)	Day 7 (s)	Day 10 (s)	Day 14 (s)
I	Normal/Sham control	8.62 ± 0.31	8.74 ± 0.29	8.86 ± 0.30	8.94 ± 0.28	9.02 ± 0.27
II	CCI control	8.58 ± 0.30	4.21 ± 0.24	4.08 ± 0.22	3.96 ± 0.21	3.84 ± 0.20
III	Thymoquinone 5 mg/kg	8.64 ± 0.29	5.02 ± 0.25	5.72 ± 0.27	6.48 ± 0.28	7.26 ± 0.30
IV	Curcumin 50 mg/kg	8.60 ± 0.32	4.86 ± 0.23	5.54 ± 0.26	6.24 ± 0.27	7.04 ± 0.29
V	Thymoquinone + Curcumin	8.63 ± 0.30	5.46 ± 0.27	6.42 ± 0.29	7.38 ± 0.31	8.18 ± 0.30
VI	Pregabalin 10 mg/kg	8.61 ± 0.28	5.58 ±	6.58 ±	7.54 ±	8.32 ±
			0.26	0.30	0.29	0.28

The CCI control group showed a decrease in reaction latency from 8.58 ± 0.30 s at baseline to 3.84 ± 0.20 s on day 14. This indicated increased thermal sensitivity following CCI induction.

Thymoquinone treatment increased the reaction latency to 7.26 ± 0.30 s on day 14, whereas curcumin increased the latency to 7.04 ± 0.29 s. The combination treatment produced a higher latency of 8.18 ± 0.30 s, which was close to the response observed with pregabalin (8.32 ± 0.28 s).

The results indicated that both thymoquinone and curcumin produced antinociceptive activity in CCI-induced neuropathic pain. The combination treatment showed a greater improvement in thermal nociceptive response than either thymoquinone or curcumin alone.

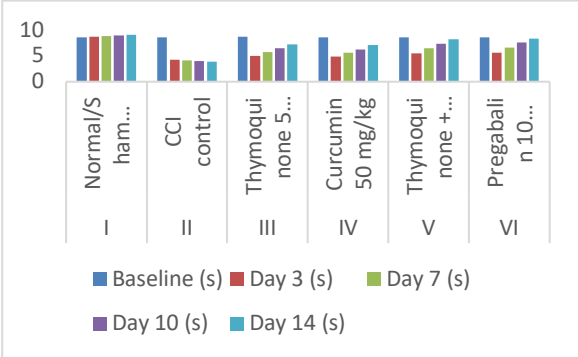


Figure 2: Effect of thymoquinone, curcumin, their combination and pregabalin on hot plate reaction latency in CCI-induced neuropathic pain.

General Observations of Experimental Animals

The general health condition of the experimental animals was monitored throughout the study. The animals were observed for changes in body weight, food intake, water intake, physical activity, grooming behaviour, general appearance and mortality. The observations were recorded during the experimental period to assess the general health and tolerability of the treatments.

A total of 36 male Wistar rats were included in the study and divided into six groups, with six animals in each group (n = 6). The animals were maintained under standard laboratory conditions and received the respective treatments for 14 consecutive days.

Effect of Treatment on Body Weight

Body weight was recorded at baseline and at the end of the treatment period. The normal/sham control animals showed a gradual increase in body weight during the study. The CCI control group showed a comparatively smaller increase in body weight, particularly during the initial period following CCI induction.

Treatment groups showed gradual maintenance and improvement in body weight during the 14-day treatment period. The combination-treated group and pregabalin-treated group showed body weight changes comparable to the normal control group.

Table 3: Effect of Treatments on Body Weight of Experimental Animals

Group	Treatment	Initial body weight (g)	Final body weight (g)	Change in body weight (g)
I	Normal/Sham control	181.67 ± 4.12	194.83 ± 4.35	+13.16
II	CCI control	180.50 ± 4.28	185.17 ± 4.11	+4.67
III	Thymoquinone 5 mg/kg	182.33 ± 3.96	191.67 ± 4.20	+9.34
IV	Curcumin 50 mg/kg	181.17 ± 4.06	190.50 ± 4.17	+9.33
V	Thymoquinone + Curcumin	180.83 ± 4.31	194.00 ± 4.46	+13.17
VI	Pregabalin 10 mg/kg	182.00 ± 4.15	193.17 ± 4.28	+11.17

The normal/sham control group showed an increase in mean body weight from 181.67 ± 4.12 g to 194.83 ± 4.35 g. In the CCI control group, body weight increased from 180.50 ± 4.28 g to 185.17 ± 4.11 g.

The thymoquinone and curcumin groups showed increases of 9.34 g and 9.33 g, respectively. The combination group showed an increase of 13.17 g, while the pregabalin group showed an increase of 11.17 g.

No marked treatment-related reduction in body weight was observed in the treatment groups.

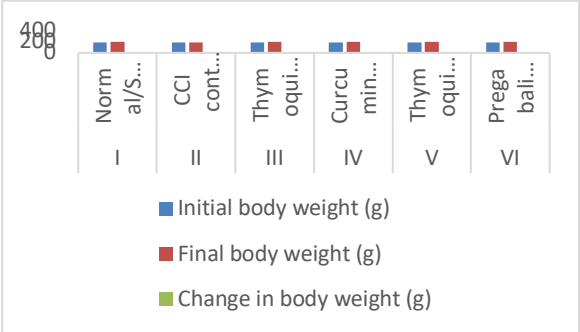


Figure 3: Effect of different treatments on initial and final body weight of experimental animals.

Food Intake

Food intake was monitored throughout the experimental period. The normal/sham control group showed regular food consumption. A mild reduction in food intake was observed in the CCI control group during the initial period following CCI induction.

The treatment groups showed gradual maintenance of food intake during the treatment period. The combination and pregabalin groups showed food intake closer to that of the normal/sham control group.

Table 4: Mean Food Intake of Experimental Animals

Group	Treatment	Mean food intake (g/animal/day)
I	Normal/Sham control	18.72 ± 0.46
II	CCI control	16.84 ± 0.51
III	Thymoquinone 5 mg/kg	18.01 ± 0.43
IV	Curcumin 50 mg/kg	17.86 ± 0.47
V	Thymoquinone + Curcumin	18.49 ± 0.44
VI	Pregabalin 10 mg/kg	18.36 ± 0.42

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The mean food intake was 18.72 ± 0.46 g/animal/day in the normal/sham control group and 16.84 ± 0.51 g/animal/day in the CCI control group. The thymoquinone, curcumin, combination and pregabalin groups showed mean food intake of 18.01 ± 0.43, 17.86 ± 0.47, 18.49 ± 0.44 and 18.36 ± 0.42 g/animal/day, respectively.
The observations indicated that the treatments did not produce any marked adverse effect on food consumption.

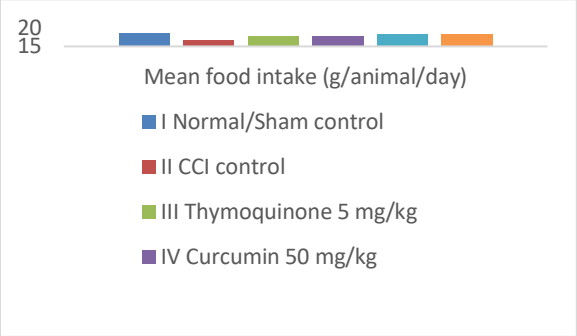


Figure 4: Mean daily food intake of experimental animals during the treatment period.

Water Intake

Water consumption was monitored throughout the experimental period. The animals in all groups continued to consume water normally. The CCI control group showed a slight reduction in water intake during the initial post-operative period, followed by gradual recovery.

Table 5: Mean Water Intake of Experimental Animals

Group	Treatment	Mean water intake (mL/animal/day)
I	Normal/Sham control	28.42 ± 0.72
II	CCI control	26.71 ± 0.68
III	Thymoquinone 5 mg/kg	27.84 ± 0.64
IV	Curcumin 50 mg/kg	27.63 ± 0.70
V	Thymoquinone + Curcumin	28.16 ± 0.66
VI	Pregabalin 10 mg/kg	28.05 ± 0.61

The mean water intake ranged from 26.71 ± 0.68 to 28.42 ± 0.72 mL/animal/day among the experimental groups. No marked difference in water consumption was observed in the treatment groups compared with the normal/sham control group.

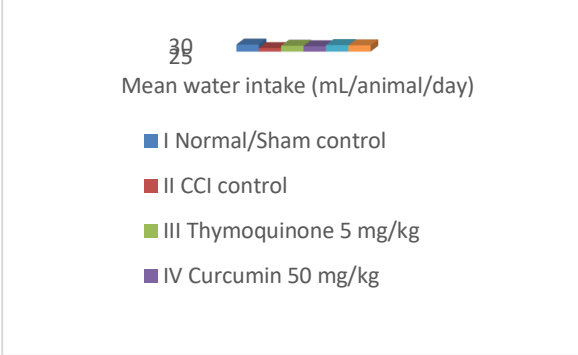


Figure 5: Mean daily water intake of experimental animals.

General Physical and Behavioural Observations

The animals were routinely observed for general appearance, locomotor activity, grooming, posture, feeding behaviour and visible signs of stress. The CCI control group showed reduced spontaneous activity and mild reduction in grooming behaviour after induction of neuropathic pain.
The thymoquinone and curcumin treatment groups showed gradual improvement in spontaneous activity and grooming behaviour during the treatment period. The combination-treated animals showed better maintenance of normal activity compared with the individual treatment groups. The pregabalin-treated group also showed improvement in general activity.
No abnormal clinical signs such as severe lethargy, marked respiratory difficulty, persistent convulsions or other severe treatment-related reactions were observed during routine observation.

Table 6: Qualitative General Observations of Experimental Animals

Observation	Group I	Group II	Group III	Group IV	Group V	Group VI
General appearance	Normal	Mildly altered	Normal	Normal	Normal	Normal

Physical activity	Normal	Reduced	Improved	Improved	Near normal	Near normal
Grooming	Normal	Slightly reduced	Improved	Improved	Near normal	Near normal
Posture	Normal	Mildly altered	Normal	Normal	Normal	Normal
Feeding behaviour	Normal	Slightly reduced initially	Normal	Normal	Normal	Normal
Signs of severe stress	Nil	Nil	Nil	Nil	Nil	Nil
Mortality	0/6	0/6	0/6	0/6	0/6	0/6

Mortality and Treatment Tolerance

No mortality was observed in any of the experimental groups during the study period. All six animals in each group survived until completion of the 14-day treatment period.

The overall survival rate was therefore 100% (36/36 animals). The test treatment groups did not show any obvious severe treatment-related abnormality during routine observation.

Table 7: Survival and Treatment Tolerance

Group	No. of animals	Animals survived	Mortality	Survival (%)
I – Normal/Sham control	6	6	0	100
II – CCI control	6	6	0	100
III – Thymoquinone	6	6	0	100
IV – Curcumin	6	6	0	100
V – Combination	6	6	0	100
VI – Pregabalin	6	6	0	100
Total	**36**	**36**	**0**	**100**

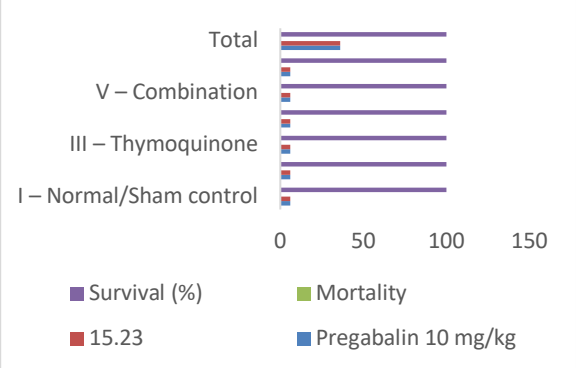


Figure 6: Survival percentage of experimental animals in all treatment groups.

Effect of CCI Induction on Neuropathic Pain

The effect of chronic constriction injury (CCI) on the development of neuropathic pain was evaluated by

assessing mechanical and thermal nociceptive responses before and after CCI induction. Mechanical sensitivity was assessed using the Von Frey test, whereas thermal nociception was assessed using the hot plate test. Baseline observations were recorded before surgical intervention and were used as reference values for evaluating the effect of CCI induction.

The experimental study included six groups, namely normal/sham control, CCI control, thymoquinone, curcumin, combination and pregabalin groups. The CCI model was used to induce neuropathic pain, followed by behavioural assessment at predetermined time points.

Baseline Von Frey Response

Before induction of CCI, the mechanical withdrawal threshold was measured in all experimental animals using the Von Frey apparatus. The baseline values were comparable among the different experimental groups, indicating similar mechanical sensitivity before induction of neuropathic pain.

The mean paw withdrawal threshold ranged between 15.17 ± 0.40 g and 15.24 ± 0.43 g across the six groups. No marked difference was observed between the groups at baseline.

Table 8: Baseline Von Frey Response Before CCI Induction

Group	Treatment	Paw withdrawal threshold (g)
I	Normal/Sham control	15.21 ± 0.42
II	CCI control	15.18 ± 0.45
III	Thymoquinone 5 mg/kg	15.24 ± 0.43
IV	Curcumin 50 mg/kg	15.17 ± 0.40
V	Thymoquinone + Curcumin	15.20 ± 0.44
VI	Pregabalin 10 mg/kg	15.23 ± 0.41

The results showed that the baseline mechanical withdrawal threshold was approximately 15 g in all groups. The small variation between groups indicated a comparable baseline mechanical nociceptive response before CCI induction.

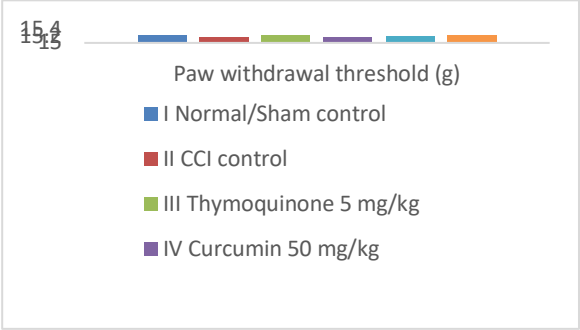


Figure 7: Baseline paw withdrawal threshold of experimental animals before CCI induction.

Post-CCI Von Frey Response

Following CCI induction, a marked reduction in paw withdrawal threshold was observed in the CCI control group compared with its baseline value. The mean threshold decreased from 15.18 ± 0.45 g before CCI to 6.82 ± 0.31 g after CCI induction, indicating the development of mechanical hypersensitivity.

The normal/sham control group maintained a high paw withdrawal threshold of 15.08 ± 0.39 g. In comparison, the CCI control group showed a pronounced reduction in mechanical threshold.

Table 9: Effect of CCI Induction on Von Frey Paw Withdrawal Threshold

Group	Treatment	Baseline (g)	Post-CCI (g)	Reduction from baseline (%)
I	Normal/Sham control	15.21 ± 0.42	15.08 ± 0.39	0.85
II	CCI control	15.18 ± 0.45	6.82 ± 0.31	55.07
III	Thymoquinone 5 mg/kg	15.24 ± 0.43	8.12 ± 0.35	46.72

IV	Curcumin 50 mg/kg	15.17 ± 0.40	7.86 ± 0.32	48.19
V	Thymoquinone + Curcumin	15.20 ± 0.44	9.14 ± 0.36	39.87
VI	Pregabalin 10 mg/kg	15.23 ± 0.41	9.36 ± 0.34	38.54

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The greatest reduction in paw withdrawal threshold was observed in the CCI control group. Compared with baseline, the post-CCI threshold decreased by approximately 55.07%.

The reduction in mechanical withdrawal threshold following CCI demonstrated the development of mechanical allodynia in the neuropathic pain model. The subsequent treatment-related improvement was evaluated during the 14-day treatment period.

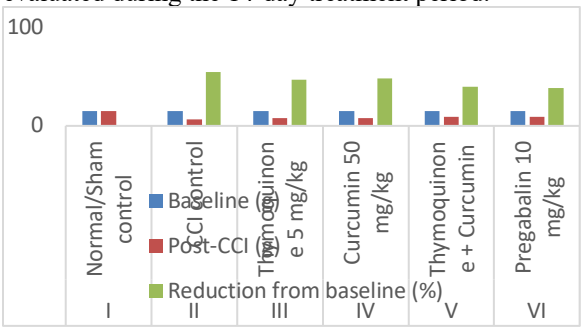


Figure 8: Comparison of baseline and post-CCI paw withdrawal threshold.

Baseline Hot Plate Reaction Time

Baseline thermal nociceptive response was evaluated using the hot plate apparatus before induction of CCI. Reaction latency was recorded as the time between placement of the animal on the hot plate and the first characteristic nociceptive response, such as paw licking, paw withdrawal or jumping.

The baseline reaction time was comparable among all experimental groups. The mean reaction latency ranged from 8.58 ± 0.30 s to 8.64 ± 0.29 s.

Table 10: Baseline Hot Plate Reaction Time Before CCI Induction

Group	Treatment	Reaction latency (s)
I	Normal/Sham control	8.62 ± 0.31
II	CCI control	8.58 ± 0.30
III	Thymoquinone 5 mg/kg	8.64 ± 0.29
IV	Curcumin 50 mg/kg	8.60 ± 0.32
V	Thymoquinone + Curcumin	8.63 ± 0.30
VI	Pregabalin 10 mg/kg	8.61 ± 0.28

The baseline reaction latency was approximately 8.6 s in all groups, indicating that the animals had comparable thermal nociceptive sensitivity before CCI induction.

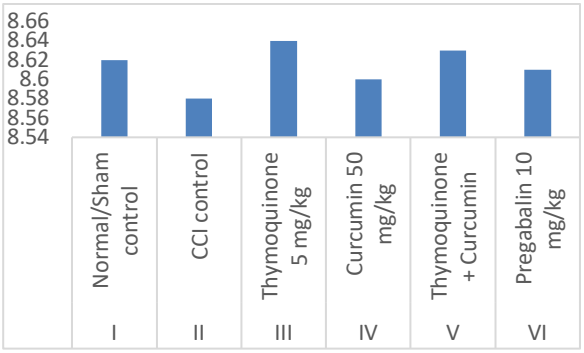


Figure 9: Baseline hot plate reaction latency before CCI induction.

Post-CCI Hot Plate Reaction Time

After induction of CCI, the CCI control group showed a marked reduction in hot plate reaction latency compared with the baseline response. The mean reaction latency decreased from 8.58 ± 0.30 s to 4.21 ± 0.24 s, indicating increased thermal sensitivity following CCI induction.

The normal/sham control group showed a comparatively stable reaction latency of 8.74 ± 0.29 s. The reduction in reaction latency was most evident in the CCI control group.

Table 11: Effect of CCI Induction on Hot Plate Reaction Latency

Group	Treatment	Baseline (s)	Post-CCI (s)	Reduction from baseline (%)

I	Normal/Sham control	8.62 ± 0.31	8.74 ± 0.29	—
II	CCI control	8.58 ± 0.30	4.21 ± 0.24	50.93
III	Thymoquinone 5 mg/kg	8.64 ± 0.29	5.02 ± 0.25	41.90
IV	Curcumin 50 mg/kg	8.60 ± 0.32	4.86 ± 0.23	43.49
V	Thymoquinone + Curcumin	8.63 ± 0.30	5.46 ± 0.27	36.73
VI	Pregabalin 10 mg/kg	8.61 ± 0.28	5.58 ± 0.26	35.19

The CCI control group showed approximately 50.93% reduction in hot plate reaction latency compared with its baseline value. This reduction indicated increased thermal nociceptive sensitivity following CCI induction.

The reduction in both mechanical withdrawal threshold and thermal reaction latency after CCI demonstrated successful induction of neuropathic pain-like behavioural changes in the experimental animals.

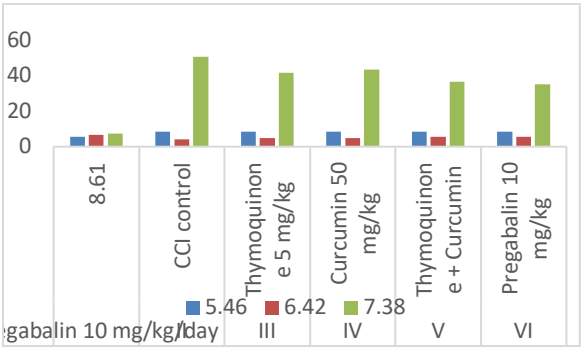


Figure 10: Comparison of baseline and post-CCI hot plate reaction latency.

Effect of Thymoquinone on CCI-Induced Neuropathic Pain

Thymoquinone was evaluated for its effect on CCI-induced neuropathic pain using the Von Frey test and hot plate test. The response of animals treated with thymoquinone (5 mg/kg/day) was compared with the CCI control group. Behavioural assessments were carried out at predetermined intervals during the 14-day treatment period.

Effect of Thymoquinone on Mechanical Allodynia by Von Frey Test

The effect of thymoquinone on mechanical allodynia was assessed using the Von Frey test. The CCI control group showed a marked reduction in paw withdrawal threshold following CCI induction, indicating the development of mechanical hypersensitivity.

The mean baseline Von Frey response in the thymoquinone-treated group was 15.24 ± 0.43 g. Following CCI induction, the response decreased to 8.12 ± 0.35 g on Day 3, confirming the development of mechanical allodynia. With continued administration of thymoquinone, a progressive increase in paw withdrawal threshold was observed.

The Von Frey response increased from 8.12 ± 0.35 g on Day 3 to 9.46 ± 0.37 g on Day 7, 10.84 ± 0.42 g on Day 10, and 12.26 ± 0.40 g on Day 14. Thus, thymoquinone produced a gradual improvement in mechanical withdrawal threshold during the treatment period.

Table 12: Effect of Thymoquinone on Mechanical Allodynia by Von Frey Test

Group	Treatment	Baseline (g)	Day 3 (g)	Day 7 (g)	Day 10 (g)	Day 14 (g)
I	Normal/Sham control	15.21 ± 0.42	15.08 ± 0.39	15.34 ± 0.41	15.46 ± 0.38	15.52 ± 0.36
II	CCI control	15.18 ± 0.45	6.82 ± 0.31	6.54 ± 0.29	6.31 ± 0.27	6.18 ± 0.25

III	Thymoquinone 5 mg/kg/day	15.24 ± 0.43	8.12 ± 0.35	9.46 ± 0.37	10.84 ± 0.42	12.26 ± 0.40
IV	Curcumin 50 mg/kg/day	15.17 ± 0.40	7.86 ± 0.32	9.18 ± 0.35	10.51 ± 0.39	11.84 ± 0.38
V	Thymoquinone + Curcumin	15.20 ± 0.44	9.14 ± 0.36	11.28 ± 0.41	12.84 ± 0.43	14.02 ± 0.39
VI	Pregabalin 10 mg/kg/day	15.23 ± 0.41	9.36 ± 0.34	11.62 ± 0.40	13.18 ± 0.42	14.28 ± 0.37

Thymoquinone treatment increased the mechanical withdrawal threshold compared with the CCI control group. On Day 14, the threshold in the thymoquinone group was 12.26 ± 0.40 g, compared with 6.18 ± 0.25 g in the CCI control group. This indicated a substantial reduction in CCI-induced mechanical hypersensitivity.

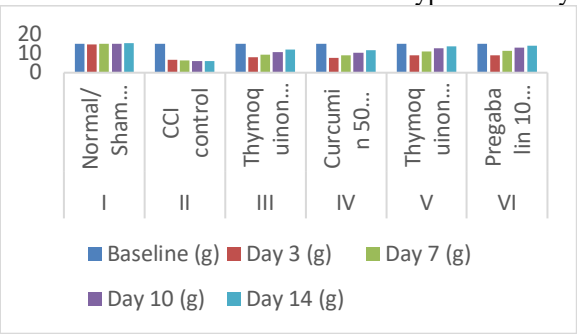


Figure 11: Effect of thymoquinone on mechanical allodynia in CCI-induced neuropathic pain using the Von Frey test

Effect of Thymoquinone on Thermal Nociception by Hot Plate Test

The effect of thymoquinone on thermal nociception was evaluated using the hot plate test. The baseline reaction latency in the thymoquinone-treated group was 8.64 ± 0.29 s. After CCI induction, the reaction latency decreased to 5.02 ± 0.25 s on Day 3, indicating increased sensitivity to the thermal stimulus.

Following treatment with thymoquinone, the reaction latency progressively increased during the study period. The mean latency increased to 5.72 ± 0.27 s on Day 7, 6.48 ± 0.28 s on Day 10, and 7.26 ± 0.30 s on Day 14.

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Table 13: Effect of Thymoquinone on Thermal Nociception by Hot Plate Test

Group	Treatment	Baseline (s)	Day 3 (s)	Day 7 (s)	Day 10 (s)	Day 14 (s)
I	Normal/Sham control	8.62 ± 0.31	8.74 ± 0.29	8.86 ± 0.30	8.94 ± 0.28	9.02 ± 0.27
II	CCI control	8.58 ± 0.30	4.21 ± 0.24	4.08 ± 0.22	3.96 ± 0.21	3.84 ± 0.20
III	Thymoquinone 5 mg/kg/day	8.64 ± 0.29	5.02 ± 0.25	5.72 ± 0.27	6.48 ± 0.28	7.26 ± 0.30
IV	Curcumin 50 mg/kg/day	8.60 ± 0.32	4.86 ± 0.23	5.54 ± 0.26	6.24 ± 0.27	7.04 ± 0.29

V	Thymoquinone + Curcumin	8.63 ± 0.30	5.46 ± 0.27	6.42 ± 0.29	7.38 ± 0.31	8.18 ± 0.30
VI	Pregabalin 10 mg/kg/day	8.61 ± 0.28	5.58 ± 0.26	6.58 ± 0.30	7.54 ± 0.29	8.32 ± 0.28

Thymoquinone produced a progressive increase in hot plate reaction latency compared with the CCI control group. On Day 14, the thymoquinone-treated group showed a reaction latency of 7.26 ± 0.30 s, whereas the CCI control group showed 3.84 ± 0.20 s. The increase in reaction latency indicated improvement in CCI-induced thermal hypersensitivity.

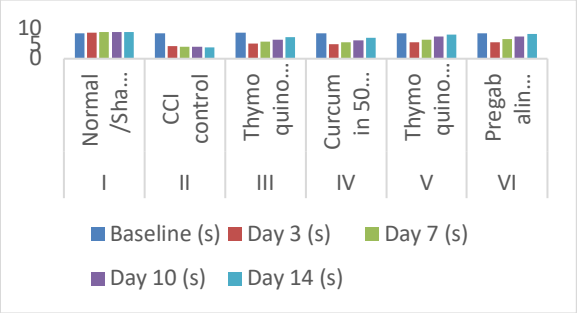


Figure 12: Effect of thymoquinone on thermal nociception in CCI-induced neuropathic pain using the hot plate test.

Effect of Curcumin on CCI-Induced Neuropathic Pain

Curcumin was evaluated for its protective and analgesic effect against CCI-induced neuropathic pain using mechanical and thermal behavioural tests. Curcumin was administered at a dose of 50 mg/kg/day, and its effect was assessed at predetermined intervals over the 14-day treatment period.

Effect of Curcumin on Mechanical Allodynia by Von Frey Test

The Von Frey test showed a marked reduction in paw withdrawal threshold after CCI induction in the curcumin-treated group. The baseline withdrawal threshold was 15.17 ± 0.40 g, which decreased to 7.86 ± 0.32 g on Day 3 following CCI induction.

After administration of curcumin, the paw withdrawal threshold gradually increased throughout the treatment period. The mean values increased from 7.86 ± 0.32 g on Day 3 to 9.18 ± 0.35 g on Day 7, 10.51 ± 0.39 g on Day 10, and 11.84 ± 0.38 g on Day 14.

Table 14: Effect of Curcumin on Mechanical Allodynia by Von Frey Test

Group	Treatment	Baseline (g)	Day 3 (g)	Day 7 (g)	Day 10 (g)	Day 14 (g)
I	Normal/Sham control	15.21 ± 0.42	15.08 ± 0.39	15.34 ± 0.41	15.46 ± 0.38	15.52 ± 0.36
II	CCI control	15.18 ± 0.45	6.82 ± 0.31	6.54 ± 0.29	6.31 ± 0.27	6.18 ± 0.25
III	Thymoquinone 5 mg/kg/day	15.24 ± 0.43	8.12 ± 0.35	9.46 ± 0.37	10.84 ± 0.42	12.26 ± 0.40
IV	Curcumin 50 mg/kg/day	15.17 ± 0.40	7.86 ± 0.32	9.18 ± 0.35	10.51 ± 0.39	11.84 ± 0.38
V	Thymoquinone + Curcumin	15.20 ± 0.44	9.14 ± 0.36	11.28 ± 0.41	12.84 ± 0.43	14.02 ± 0.39

VI	Pregabalin 10 mg/kg/day	15.23 ± 0.41	9.36 ± 0.34	11.62 ± 0.40	13.18 ± 0.42	14.28 ± 0.37

Curcumin treatment resulted in a progressive improvement in mechanical withdrawal threshold compared with the CCI control group. On Day 14, the mean threshold increased to 11.84 ± 0.38 g in the curcumin-treated group compared with 6.18 ± 0.25 g in the CCI control group.

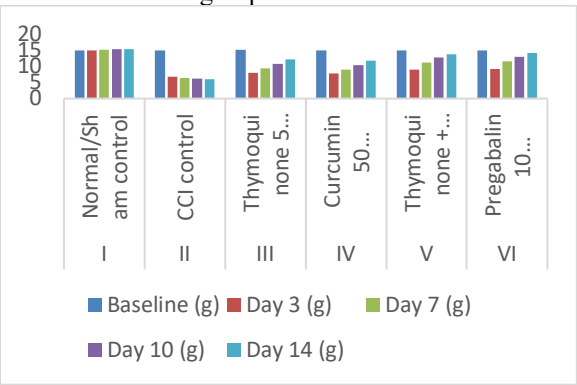


Figure 13: Effect of curcumin on mechanical allodynia in CCI-induced neuropathic pain using the Von Frey test..

Effect of Curcumin on Thermal Nociception by Hot Plate Test

The effect of curcumin on thermal nociception was determined by measuring the reaction latency in the hot plate test. The baseline reaction latency in the curcumin-treated group was 8.60 ± 0.32 s. Following CCI induction, the reaction latency decreased to 4.86 ± 0.23 s on Day 3, indicating increased thermal sensitivity.

Treatment with curcumin resulted in a gradual increase in reaction latency during the 14-day treatment period. The mean reaction latency increased to 5.54 ± 0.26 s on Day 7, 6.24 ± 0.27 s on Day 10, and 7.04 ± 0.29 s on Day 14.

Table 15: Effect of Curcumin on Thermal Nociception by Hot Plate Test

Group	Treatment	Baseline (s)	Day 3 (s)	Day 7 (s)	Day 10 (s)	Day 14 (s)
I	Normal/Sham control	8.62 ± 0.31	8.74 ± 0.29	8.86 ± 0.30	8.94 ± 0.28	9.02 ± 0.27
II	CCI control	8.58 ± 0.30	4.21 ± 0.24	4.08 ± 0.22	3.96 ± 0.21	3.84 ± 0.20
III	Thymoquinone 5 mg/kg/day	8.64 ± 0.29	5.02 ± 0.25	5.72 ± 0.27	6.48 ± 0.28	7.26 ± 0.30
IV	Curcumin 50 mg/kg/day	8.60 ± 0.32	4.86 ± 0.23	5.54 ± 0.26	6.24 ± 0.27	7.04 ± 0.29
V	Thymoquinone + Curcumin	8.63 ± 0.30	5.46 ± 0.27	6.42 ± 0.29	7.38 ± 0.31	8.18 ± 0.30
VI	Pregabalin 10 mg/kg/day	8.61 ± 0.28	5.58 ± 0.26	6.58 ± 0.30	7.54 ± 0.29	8.32 ± 0.28

Curcumin produced a gradual increase in hot plate reaction latency compared with the CCI control group. On Day 14, the reaction latency was 7.04 ± 0.29 s in the curcumin group compared with 3.84 ± 0.20 s in the

CCI control group. This indicated an improvement in CCI-induced thermal hypersensitivity.

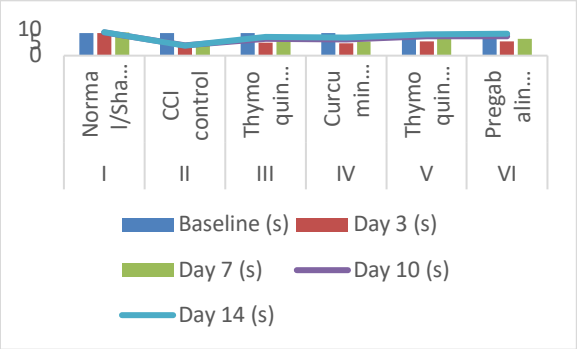


Figure 14: Effect of curcumin on thermal nociception in CCI-induced neuropathic pain using the hot plate test

Effect of Combined Thymoquinone and Curcumin Treatment

The combined effect of thymoquinone and curcumin was evaluated in Group V animals with CCI-induced neuropathic pain. Animals received thymoquinone at a dose of 5 mg/kg/day by the intraperitoneal route together with curcumin at a dose of 50 mg/kg/day by the oral route for 14 consecutive days. The combination treatment was included to determine whether simultaneous administration of both compounds produced greater improvement in mechanical and thermal nociceptive responses than the individual treatments.

Behavioural responses were assessed using the Von Frey test for mechanical allodynia and the hot plate test for thermal nociception. Baseline observations were recorded before CCI induction, followed by repeated assessments on Days 3, 7, 10 and 14.

Effect of Combination Treatment on Mechanical Allodynia

The effect of combined thymoquinone and curcumin treatment on mechanical allodynia was assessed using the Von Frey test. The combination-treated animals showed a marked reduction in paw withdrawal threshold after CCI induction, followed by a progressive increase during the treatment period.

The baseline paw withdrawal threshold in the combination group was 15.20 ± 0.44 g. After CCI induction, the threshold decreased to 9.14 ± 0.36 g on Day 3, indicating the development of mechanical hypersensitivity. With continued administration of the combination treatment, the withdrawal threshold increased to 11.28 ± 0.41 g on Day 7, 12.84 ± 0.43 g on Day 10, and 14.02 ± 0.39 g on Day 14.

The Day 14 value in the combination group was substantially higher than the corresponding value of 6.18 ± 0.25 g observed in the CCI control group. The combination treatment therefore showed a progressive improvement in mechanical withdrawal threshold over the treatment period.

Table 16: Effect of Combined Thymoquinone and Curcumin Treatment on Mechanical Allodynia by Von Frey Test

Group	Treatment	Baseline (g)	Day 3 (g)	Day 7 (g)	Day 10 (g)	Day 14 (g)
I	Normal/Sham control	15.21 ± 0.42	15.08 ± 0.39	15.34 ± 0.41	15.46 ± 0.38	15.52 ± 0.36
II	CCI control	15.18 ± 0.45	6.82 ± 0.31	6.54 ± 0.29	6.31 ± 0.27	6.18 ± 0.25
III	Thymoquinone 5 mg/kg/day	15.24 ± 0.43	8.12 ± 0.35	9.46 ± 0.37	10.84 ± 0.42	12.26 ± 0.40
IV	Curcumin 50 mg/kg/day	15.17 ± 0.40	7.86 ± 0.32	9.18 ± 0.35	10.51 ± 0.39	11.84 ± 0.38
V	Thymoquinone + Curcumin	15.20 ± 0.44	9.14 ± 0.36	11.28 ± 0.41	12.84 ± 0.43	14.02 ± 0.39

VI	Pregabalin 10 mg/kg/day	15.23 ± 0.41	9.36 ± 0.34	11.62 ± 0.40	13.18 ± 0.42	14.28 ± 0.37

The combination treatment produced a progressive increase in paw withdrawal threshold from 9.14 ± 0.36 g on Day 3 to 14.02 ± 0.39 g on Day 14. In comparison, the CCI control group showed a further decrease in withdrawal threshold from 6.82 ± 0.31 g to 6.18 ± 0.25 g over the same period. The Day 14 response of the combination group was close to the sham value of 15.52 ± 0.36 g and the pregabalin value of 14.28 ± 0.37 g.

The improvement observed with the combination was greater than that observed with thymoquinone or curcumin alone. On Day 14, thymoquinone and curcumin produced withdrawal thresholds of 12.26 ± 0.40 g and 11.84 ± 0.38 g, respectively, whereas the combination treatment produced 14.02 ± 0.39 g.

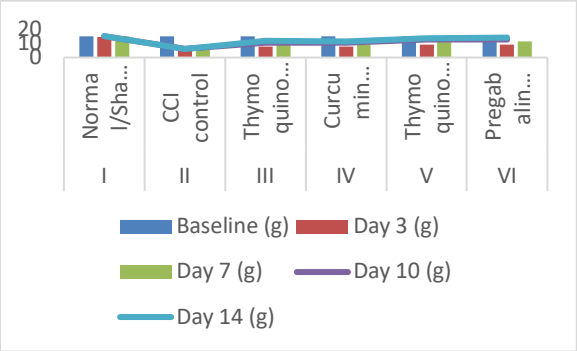


Figure 15: Effect of combined thymoquinone and curcumin treatment on mechanical allodynia in CCI-induced neuropathic pain.

Effect of Combination Treatment on Thermal Nociception

The effect of combined thymoquinone and curcumin treatment on thermal nociception was evaluated using the hot plate test. The baseline reaction latency in the combination group was 8.63 ± 0.30 s. Following CCI induction, the reaction latency decreased to 5.46 ± 0.27 s on Day 3, indicating increased sensitivity to the thermal stimulus.

A progressive increase in reaction latency was observed following combination treatment. The reaction latency increased to 6.42 ± 0.29 s on Day 7, 7.38 ± 0.31 s on Day 10, and 8.18 ± 0.30 s on Day 14. The combination group showed a considerably higher reaction latency than the CCI control group, which showed a reaction latency of 3.84 ± 0.20 s on Day 14. The findings indicated improvement in thermal hypersensitivity following combined treatment.

Table 17: Effect of Combined Thymoquinone and Curcumin Treatment on Thermal Nociception by Hot Plate Test

Group	Treatment	Baseline (s)	Day 3 (s)	Day 7 (s)	Day 10 (s)	Day 14 (s)
I	Normal/Sham control	8.62 ± 0.31	8.74 ± 0.29	8.86 ± 0.30	8.94 ± 0.28	9.02 ± 0.27
II	CCI control	8.58 ± 0.30	4.21 ± 0.24	4.08 ± 0.22	3.96 ± 0.21	3.84 ± 0.20
III	Thymoquinone 5 mg/kg/day	8.64 ± 0.29	5.02 ± 0.25	5.72 ± 0.27	6.48 ± 0.28	7.26 ± 0.30
IV	Curcumin 50 mg/kg/day	8.60 ± 0.32	4.86 ± 0.23	5.54 ± 0.26	6.24 ± 0.27	7.04 ± 0.29
V	Thymoquinone + Curcumin	8.63 ± 0.30	5.46 ± 0.27	6.42 ± 0.29	7.38 ± 0.31	8.18 ± 0.30

VI	Pregabalin 10 mg/kg/day	8.61 ± 0.28	5.58 ± 0.26	6.58 ± 0.30	7.54 ± 0.29	8.32 ± 0.28

Combined treatment produced a progressive increase in hot plate reaction latency during the treatment period. The latency increased from 5.46 ± 0.27 s on Day 3 to 8.18 ± 0.30 s on Day 14. In contrast, the CCI control group showed a decrease from 4.21 ± 0.24 s on Day 3 to 3.84 ± 0.20 s on Day 14.

On Day 14, the combination group showed a reaction latency of 8.18 ± 0.30 s, which was higher than the values observed in the thymoquinone group (7.26 ± 0.30 s) and curcumin group (7.04 ± 0.29 s). The response was also close to that observed with pregabalin (8.32 ± 0.28 s).

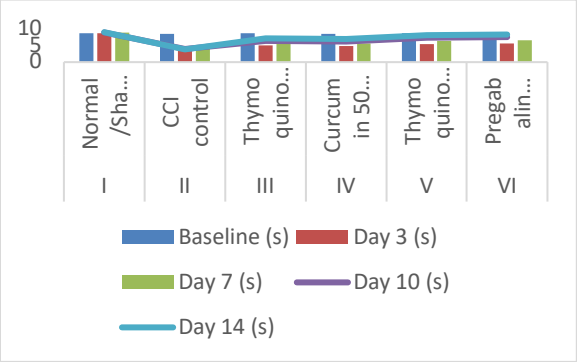


Figure 16: Effect of combined thymoquinone and curcumin treatment on thermal nociception in CCI-induced neuropathic pain.

Effect of Standard Drug Pregabalin on CCI-Induced Neuropathic Pain

Pregabalin was used as the standard drug for comparison of the antinociceptive effects of thymoquinone, curcumin and their combination in CCI-induced neuropathic pain. Pregabalin was administered at a dose of 10 mg/kg/day by the oral route for 14 consecutive days.

Effect of Pregabalin on Mechanical Allodynia

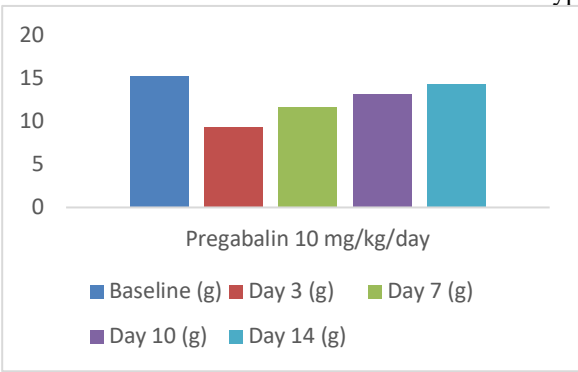
The effect of pregabalin on mechanical allodynia was assessed using the Von Frey test. The baseline paw withdrawal threshold in the pregabalin-treated group was 15.23 ± 0.41 g. Following CCI induction, the threshold decreased to 9.36 ± 0.34 g on Day 3, indicating the development of mechanical hypersensitivity.

Following treatment with pregabalin, a progressive increase in paw withdrawal threshold was observed. The mean values increased to 11.62 ± 0.40 g on Day 7, 13.18 ± 0.42 g on Day 10, and 14.28 ± 0.37 g on Day 14.

Table 18: Effect of Pregabalin on Mechanical Allodynia by Von Frey Test

Treatment group	Baseline (g)	Day 3 (g)	Day 7 (g)	Day 10 (g)	Day 14 (g)
Pregabalin 10 mg/kg/day	15.23 ± 0.41	9.36 ± 0.34	11.62 ± 0.40	13.18 ± 0.42	14.28 ± 0.37

Pregabalin produced a progressive increase in mechanical withdrawal threshold throughout the treatment period. On Day 14, the paw withdrawal threshold increased to 14.28 ± 0.37 g, compared with 6.18 ± 0.25 g in the CCI control group. The response was close to the sham-control value of 15.52 ± 0.36 g, indicating marked improvement in CCI-induced mechanical hyp



ersensitivity.

Figure 17: Effect of pregabalin on mechanical allodynia in CCI-induced neuropathic pain.

Effect of Pregabalin on Thermal Nociception

The effect of pregabalin on thermal nociception was assessed using the hot plate test. The baseline reaction latency in the pregabalin group was 8.61 ± 0.28 s. After CCI induction, the reaction latency decreased to 5.58 ± 0.26 s on Day 3.

Following treatment with pregabalin, reaction latency progressively increased to 6.58 ± 0.30 s on Day 7, 7.54 ± 0.29 s on Day 10, and 8.32 ± 0.28 s on Day 14.

Table 19: Effect of Pregabalin on Thermal Nociception by Hot Plate Test

Treatment group	Baseline (s)	Day 3 (s)	Day 7 (s)	Day 10 (s)	Day 14 (s)

Pregabalin 10 mg/kg/day	8.61 ± 0.28	5.58 ± 0.26	6.58 ± 0.30	7.54 ± 0.29	8.32 ± 0.28

Pregabalin increased the hot plate reaction latency throughout the treatment period. On Day 14, the reaction latency was 8.32 ± 0.28 s, compared with 3.84 ± 0.20 s in the CCI control group. The value was close to the sham-control response of 9.02 ± 0.27 s.

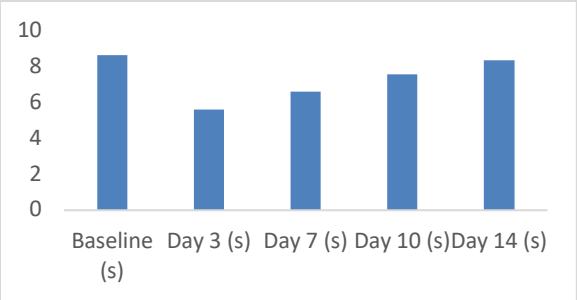


Figure 18: Effect of pregabalin on thermal nociception in CCI-induced neuropathic pain.

Comparative Effect of Thymoquinone, Curcumin and Combination Treatment

The effects of thymoquinone, curcumin and their combination were compared to determine the relative improvement in mechanical and thermal nociceptive responses following CCI. The comparison was primarily based on the behavioural responses recorded during the 14-day treatment period.

Comparison of Von Frey Response Among Treatment Groups

The Von Frey test demonstrated a progressive increase in paw withdrawal threshold in all three treatment groups following CCI induction. However, the magnitude of improvement differed between the individual and combination treatments.

On Day 14, the paw withdrawal threshold was 12.26 ± 0.40 g in the thymoquinone group and 11.84 ± 0.38 g in the curcumin group. The combination group showed a higher withdrawal threshold of 14.02 ± 0.39 g.

Table 20: Comparative Effect of Treatments on Von Frey Paw Withdrawal Threshold

Treatment	Day 3 (g)	Day 7 (g)	Day 10 (g)	Day 14 (g)
CCI control	6.82 ± 0.31	6.54 ± 0.29	6.31 ± 0.27	6.18 ± 0.25
Thymoquinone 5 mg/kg/day	8.12 ± 0.35	9.46 ± 0.37	10.84 ± 0.42	12.26 ± 0.40
Curcumin 50 mg/kg/day	7.86 ± 0.32	9.18 ± 0.35	10.51 ± 0.39	11.84 ± 0.38
Combination	9.14 ± 0.36	11.28 ± 0.41	12.84 ± 0.43	14.02 ± 0.39
Pregabalin 10 mg/kg/day	9.36 ± 0.34	11.62 ± 0.40	13.18 ± 0.42	14.28 ± 0.37

All treatment groups showed improvement compared with the CCI control group. The combination treatment produced a greater increase in paw withdrawal threshold than either thymoquinone or curcumin alone. The Day 14 response of the combination group (14.02 ± 0.39 g) was close to that of pregabalin (14.28 ± 0.37 g).

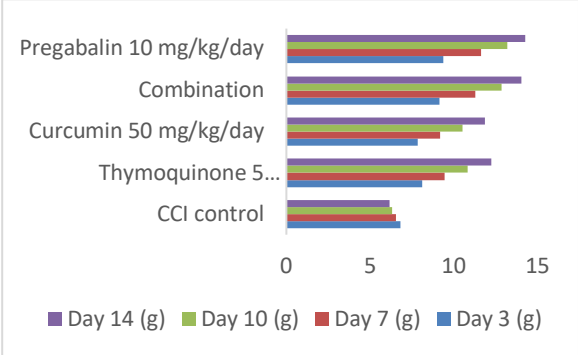


Figure 19: Comparative effect of thymoquinone, curcumin, combination treatment and pregabalin on Von Frey paw withdrawal threshold.

Comparison of Hot Plate Reaction Time Among Treatment Groups

The hot plate test also showed progressive improvement following treatment. The CCI control group showed a continued reduction in reaction latency, whereas the thymoquinone, curcumin, combination and pregabalin groups showed progressive increases.

On Day 14, the reaction latency was 7.26 ± 0.30 s with thymoquinone, 7.04 ± 0.29 s with curcumin, 8.18 ± 0.30 s with the combination treatment and 8.32 ± 0.28 s with pregabalin.

Table 21: Comparative Effect of Treatments on Hot Plate Reaction Latency

Treatment	Day 3 (s)	Day 7 (s)	Day 10 (s)	Day 14 (s)
CCI control	4.21 ± 0.24	4.08 ± 0.22	3.96 ± 0.21	3.84 ± 0.20
Thymoquinone 5 mg/kg/day	5.02 ± 0.25	5.72 ± 0.27	6.48 ± 0.28	7.26 ± 0.30
Curcumin 50 mg/kg/day	4.86 ± 0.23	5.54 ± 0.26	6.24 ± 0.27	7.04 ± 0.29
Combination	5.46 ± 0.27	6.42 ± 0.29	7.38 ± 0.31	8.18 ± 0.30
Pregabalin 10 mg/kg/day	5.58 ± 0.26	6.58 ± 0.30	7.54 ± 0.29	8.32 ± 0.28

Thymoquinone, curcumin, combination treatment and pregabalin all produced higher reaction latency than the CCI control group. The combination treatment showed a greater increase than the individual treatments, while the response remained close to that produced by pregabalin.

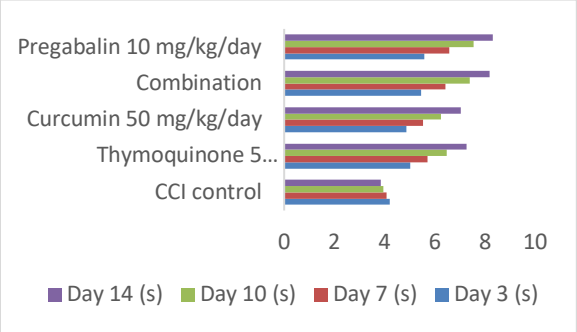


Figure 20: Comparative effect of thymoquinone, curcumin, combination treatment and pregabalin on hot plate reaction latency.

Comparison of Test Treatments with Standard Drug

The effects of thymoquinone, curcumin and their combination were compared with pregabalin, which was used as the standard treatment. The comparison was based on the Day 14 behavioural responses because this represented the response after completion of the 14-day treatment period.

Comparison of Thymoquinone with Pregabalin

On Day 14, the thymoquinone-treated group showed a Von Frey withdrawal threshold of 12.26 ± 0.40 g, whereas the pregabalin-treated group showed 14.28 ± 0.37 g. Thus, the response with thymoquinone was lower than that observed with pregabalin.

In the hot plate test, thymoquinone produced a reaction latency of 7.26 ± 0.30 s, compared with 8.32 ± 0.28 s with pregabalin.

Table 22: Comparison of Thymoquinone with Pregabalin on Day 14

Parameter	Thymoquinone	Pregabalin
Von Frey withdrawal threshold (g)	12.26 ± 0.40	14.28 ± 0.37
Hot plate reaction latency (s)	7.26 ± 0.30	8.32 ± 0.28

Thymoquinone produced a marked improvement in both mechanical and thermal nociceptive responses, although the response was lower than that observed with pregabalin.

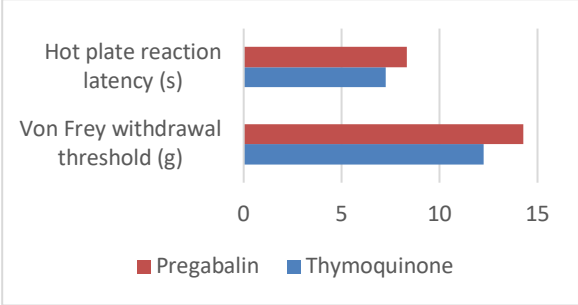


Figure 21: Comparison of thymoquinone and pregabalin on Day 14 Von Frey withdrawal threshold and hot plate reaction latency.

Comparison of Curcumin with Pregabalin

On Day 14, the curcumin group showed a Von Frey withdrawal threshold of 11.84 ± 0.38 g, compared with 14.28 ± 0.37 g in the pregabalin group. Similarly, the hot plate reaction latency was 7.04 ± 0.29 s with curcumin and 8.32 ± 0.28 s with pregabalin.

Table 23: Comparison of Curcumin with Pregabalin on Day 14

Parameter	Curcumin	Pregabalin
Von Frey withdrawal threshold (g)	11.84 ± 0.38	14.28 ± 0.37
Hot plate reaction latency (s)	7.04 ± 0.29	8.32 ± 0.28

Curcumin produced an improvement in both mechanical allodynia and thermal hypersensitivity. However, the observed responses were lower than those produced by pregabalin.

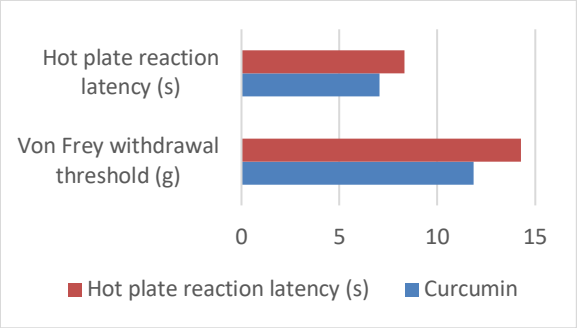


Figure 22: Comparison of curcumin and pregabalin on Day 14 Von Frey withdrawal threshold and hot plate reaction latency.

Comparison of Combination Treatment with Pregabalin

The combination treatment showed responses that were close to those observed with pregabalin. On Day 14, the Von Frey withdrawal threshold was 14.02 ± 0.39 g in the combination group and 14.28 ± 0.37 g in the pregabalin group.

Similarly, the hot plate reaction latency was 8.18 ± 0.30 s in the combination group compared with 8.32 ± 0.28 s in the pregabalin group.

Table 24: Comparison of Combination Treatment with Pregabalin on Day 14

Parameter	Combination	Pregabalin
Von Frey withdrawal threshold (g)	14.02 ± 0.39	14.28 ± 0.37
Hot plate reaction latency (s)	8.18 ± 0.30	8.32 ± 0.28

The combination treatment produced behavioural responses close to those observed with pregabalin. The difference between the combination and pregabalin groups was relatively small for both Von Frey withdrawal threshold and hot plate reaction latency.

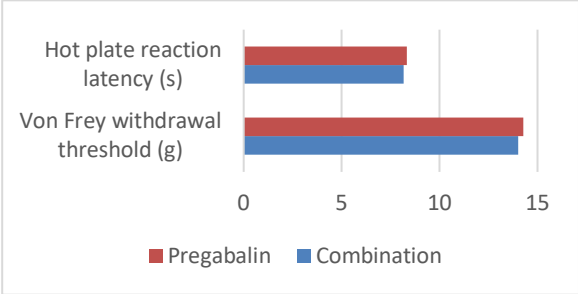


Figure 23: Comparison of combination treatment and pregabalin on Day 14 Von Frey withdrawal threshold and hot plate reaction latency.

SUMMARY AND CONCLUSION

Summary

Neuropathic pain is a chronic pain condition associated with abnormal sensory processing and is commonly characterised by mechanical allodynia and thermal hypersensitivity. The present study was undertaken to investigate the antinociceptive effects of thymoquinone and curcumin, individually and in combination, in a chronic constriction injury (CCI)-induced model of neuropathic pain in male Wistar rats. Pregabalin was used as the standard drug for comparative evaluation.

The experimental study was conducted using 36 healthy male Wistar rats, which were randomly divided into six groups of six animals each. The groups included normal/sham control, CCI control, thymoquinone-treated, curcumin-treated, combination-treated and pregabalin-treated groups. Treatment was continued for 14 consecutive days. Mechanical nociceptive responses were assessed using the Von Frey test, while thermal nociceptive responses were evaluated using the hot plate test.

Following CCI induction, the CCI control group showed a marked reduction in paw withdrawal threshold, from 15.18 ± 0.45 g at baseline to 6.18 ± 0.25 g on day 14. Similarly, the hot plate reaction latency decreased from 8.58 ± 0.30 seconds to 3.84 ± 0.20 seconds, indicating increased mechanical and thermal sensitivity.

Treatment with thymoquinone at 5 mg/kg/day improved both behavioural responses. On day 14, the paw withdrawal threshold increased to 12.26 ± 0.40 g, while the hot plate reaction latency reached 7.26 ± 0.30 seconds. Curcumin at 50 mg/kg/day also produced improvement, with a paw withdrawal threshold of 11.84 ± 0.38 g and a hot plate reaction latency of 7.04 ± 0.29 seconds.

The combined administration of thymoquinone and curcumin produced a greater improvement than either compound alone. On day 14, the combination-treated group showed a paw withdrawal threshold of 14.02 ±

0.39 g and a hot plate reaction latency of 8.18 ± 0.30 seconds. These responses were close to those observed with pregabalin, which produced values of 14.28 ± 0.37 g and 8.32 ± 0.28 seconds, respectively.

General health parameters, including body weight, food intake and water intake, were monitored throughout the study. No mortality was recorded during the 14-day treatment period, and all 36 animals survived until the completion of the experiment. No severe treatment-related clinical abnormalities were reported.

Conclusion

The present experimental study demonstrated that thymoquinone and curcumin possess antinociceptive activity in chronic constriction injury-induced neuropathic pain in male Wistar rats. Both compounds improved mechanical and thermal nociceptive responses when administered individually. However, the combined administration of thymoquinone and curcumin produced a greater improvement than either compound alone.

The response obtained with the combination treatment was close to that observed with pregabalin, the standard drug. These findings suggest that the combined use of thymoquinone and curcumin may be a promising approach for further investigation in neuropathic pain management. Nevertheless, additional studies are required to establish the underlying mechanisms, optimal dosage, long-term safety and potential clinical relevance of this combination. The present findings are limited to an experimental animal model and cannot be directly extrapolated to humans.

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