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Role of Kaempferol in CCI Induced Neuropathic Pain in Rats

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ABSTRACT

The present study, designed to investigate the role of kaempferol in the management of chronic constriction injury (CCI) of sciatic nerve induced neuropathic pain in rat. The neuropathic pain was induced by four loose ligation of right sciatic nerve (*i.e.*, CCI) in rat. The series of behavioral tests i.e., Von Frey hair filament, Hargreaves, pin prick, D'Aemour and Smith test, tail pinch tests were performed to assess the degree of mechanical allodynia, thermal hyperalgesia and mechanical hyperalgesia in paw and tail respectively. In addition, the biochemical tests *i.e.*, thiobarbituric acid reactive substances (TBARS), reduced glutathione (GSH) and total protein were also estimated in sciatic nerve tissue sample. The administration of kaempferol (50 to 100 mg/kg, *p.o.*) for 16 consecutive days significantly attenuated CCI induced rise in peripheral as well as central (thermal and mechanical) pain sensitivity. Further, it also produces the ameliorative effect on CCI induced raise in TBARS and decrease in GSH levels when compared to sham control group. Treatment of gabapentin (10 mg/kg, *p.o.* for 16 consecutive days) also produced similar effects. Hence, it may be concluded that, kaempferol may be a useful medicine for the management of neuropathic pain.

Key words: Constriction injury, Flavonoid, Kaempferol, Neuropathic pain, Sciatic nerve.

Introduction

Neuropathic pain is a chronic maladaptive neurodegenerative disorder. The damage of nervous system leads to increase in the sensitization of peripheral and central neurological systems along with denervation supersensitivity and loss of inhibitory controls (Hussain et al., 2022). Numerous conventional medicines are used for the management of neuropathic pain like anti-convulsants i.e., carbamazepine, gabapentin, pregabalin and lamotrigine; anti-depressants *i.e.*, amitryptyline, nortriptyline, lofepramine duloxetine and venlafaxine; N-methyl-D-aspartate (NMDA) receptor antagonist i.e., ketamine, methadone, amantadine, dextromethorphan and memantine; opioids *i.e.*, morphine, oxycodone, propoxyphene; topical agents *i.e.*, tramadol, codeine and dihydrocodeine. Whereas, these agents has been reported to produce the serious toxic and side effects due to chronic administration schedule in neuropathic patients (Bharti et al., 2021). Currently various herbal approach proved that, phytomedicines such as curcumin, sodium ferulate, safranal, (-)-clausenamide, tanshinone IIA, geniposidic Acid, berberine, huperzine A, limonoids and vitamin E are play a promising role in the amelioration of neurodegeneration and neuropathic pain (Dhir et al., 2020). Kaempferol is one of the most common flavonoid and exhibited various neuroprotective pharmacological activities in experimental animals (Jin et al., 2023). Based on this review of literature, this research work is attempted to explore the role of kaempferol in the chronic constriction injury (CCI) of sciatic nerve induced neuropathic pain in rats.

MATERIALS AND METHODS

Animals

Male SD rat weighing 200-250 gm were procured from Akal College of Pharmacy and Technical Education, Mastuana Sahib, Sangrur. The experimental protocol was approved by Institutional Animal Ethics Committee (IAEC No.: ATRC/31/23; Dated: 26/12/2023).

Induction of peripheral neuropathy by chronic constriction injury (CCI) of sciatic nerve

Neuropathic pain was induced in animals by CCI of sciatic nerve as described by Bennett and Xie (1988). Behavioural parameters were assessed on different time intervals *i.e.*, 0, 4, 8, 12 and 16th day. In each day, behavioural observation was performed between 09.00 am to 03.00 pm. The order of behavioural observation was performed from low intense stimuli to high intense stimuli (allodynia followed by hyperalgesia) in paw as well as in tail using various well designed protocols such as Von Frey hair filament test (Chaplan *et al.* 1994); Peripheral thermal hyperalgesic test (Hargreaves *et al.* 1988); Pin prick test (Erichsen and Blackburn-Munro, 2002); D'Aemour and Smith test (D'Amour and Smith, 1941) and Tail pinch test (Takagi *et al.* 1966).

Biochemical analysis

All the groups of animals were sacrificed after 16th day of behavioural observation by cervical dislocation and complete right sciatic nerves were isolated immediately. All part of nerves was used for the biochemical estimations. The sciatic nerve was homogenated (10 % w/v) with phosphate buffer (pH 7.4) and centrifuged at 3500 rpm for 10 min. The supernatant was used for the estimation of tissue thiobarbituric acid reactive substances (TBARS) (Ohkawa *et al.* (1979), reduced glutathione (GSH) (Ellman, 1959) and total protein levels (Lowry's *et al*. 1951).

Experimental protocol

In the present investigations, the animals were divided into four groups, each group comprising of six male SD rats.

Group I (CCI + Control): Control group received vehicle (2.5 ml, *p.o.*) for 15 consecutive days; Group II (CCI + Gabapentin): Standard group received gabapentin (10 mg/kg, p.o.) for 15 consecutive days. Group III (CCI + kaempferol): Test group received kaempferol (50 mg/kg, p.o.) for 15 consecutive days and Group IV (CCI + kaempferol): Test group received kaempferol (100 mg/kg, p.o.) for 15 consecutive days.

Statistical analysis

All results were expressed as mean standard deviation (mean ± SD). Data obtained from behavioral tests were statistically analyzed using two-way analysis of variance (ANOVA) followed by Student-Newman- Keul's test using Graph pad prism Version-5.0 software. A probability value of less than 0.05 ($P < 0.05$) was considered to be statistically significant.

RESULTS AND DISCUSSION

Effect of kaempferol on Von Frey hair filament test

When compared to a sham control group, CCI of the sciatic nerve caused a considerable development of peripheral mechanical allodynia, as seen by a rise in the percentage paw withdrawal reaction. When kaempferol (50–100 mg/kg, p.o.) is administered for 16 days in a row, the CCI-induced rise in peripheral mechanical nociceptive pain perception is considerably ($P < 0.05$) attenuated. The results of gabapentin treatment were likewise comparable. Nevertheless, neither the ruper se nor the gabapentin per se treated groups demonstrated a discernible alteration in the mechanical allodynia in rats generated by CCI (Figure 1).

Effect of kaempferol on Hargreaves test

When compared to a sham control group, CCI of the sciatic nerve caused a considerable development of thermal hyperalgesia as evidenced by a decrease in the right hind paw withdrawal threshold. The CCI-induced decrease in the thermal nociceptive pain threshold is significantly ($P < 0.05$) attenuated in a dose-dependent manner by the administration of kaempferol mg/kg, p.o. for 16 consecutive days. The results of gabapentin treatment were likewise comparable. Nevertheless, the CCI-induced thermal hyperalgesia in rats did not significantly alter in the groups treated with kaempferol and gabapentin alone (Figure 2).

Effect of kaempferol on pin prick group test

When compared to a sham control group, CCI of the sciatic nerve caused a considerable development of peripheral mechanical hyperalgesia as evidenced by an increase in the proportion of paw withdrawal reaction. In a dose-dependent manner, kaempferol administration (50 to 100 mg/kg, p.o.) for 16 consecutive days significantly ($P < 0.05$) attenuates the CCI-induced elevation of peripheral mechanical nociceptive pain sensation. The results of gabapentin treatment were likewise comparable. Nevertheless, no appreciable alterations in CCI-induced mechanical hyperalgesia in rats were observed in the groups treated with kaempferol and gabapentin alone (Figure 3).

Effect of kaempferol on *D'Aemour and Smith* test

When compared to a sham control group, CCI of the sciatic nerve caused a considerable development of thermal hyperalgesia as indicated by a decrease in tail withdrawal threshold. In a dose-dependent manner, kaempferol administration (50–100 mg/kg, p.o.) significantly ($P < 0.05$) mitigated the decrease in the thermal nociceptive pain threshold generated by CCI. Gabapentin therapy likewise has comparable outcomes. Nonetheless, neither the kaempferol nor the gabapentin treated groups significantly altered the rat's CCI-induced thermal hyperalgesia (Figure 4).

Effect of kaempferol on tail pinch test

When compared to a sham control group, CCI of the sciatic nerve caused a considerable development of central mechanical hyperalgesia as seen by an increase in the frequency of dislodgements attempts. In a dose-dependent manner, kaempferol administration (50–100 mg/kg, p.o.) reduced the increase in central mechanical nociceptive pain perception brought on by CCI. The results of gabapentin treatment were likewise comparable. Nevertheless, there were no notable ($P < 0.05$) alterations in CCI-induced mechanical hyperalgesia in the groups treated with kaempferol and gabapentin per se (Figure 5).

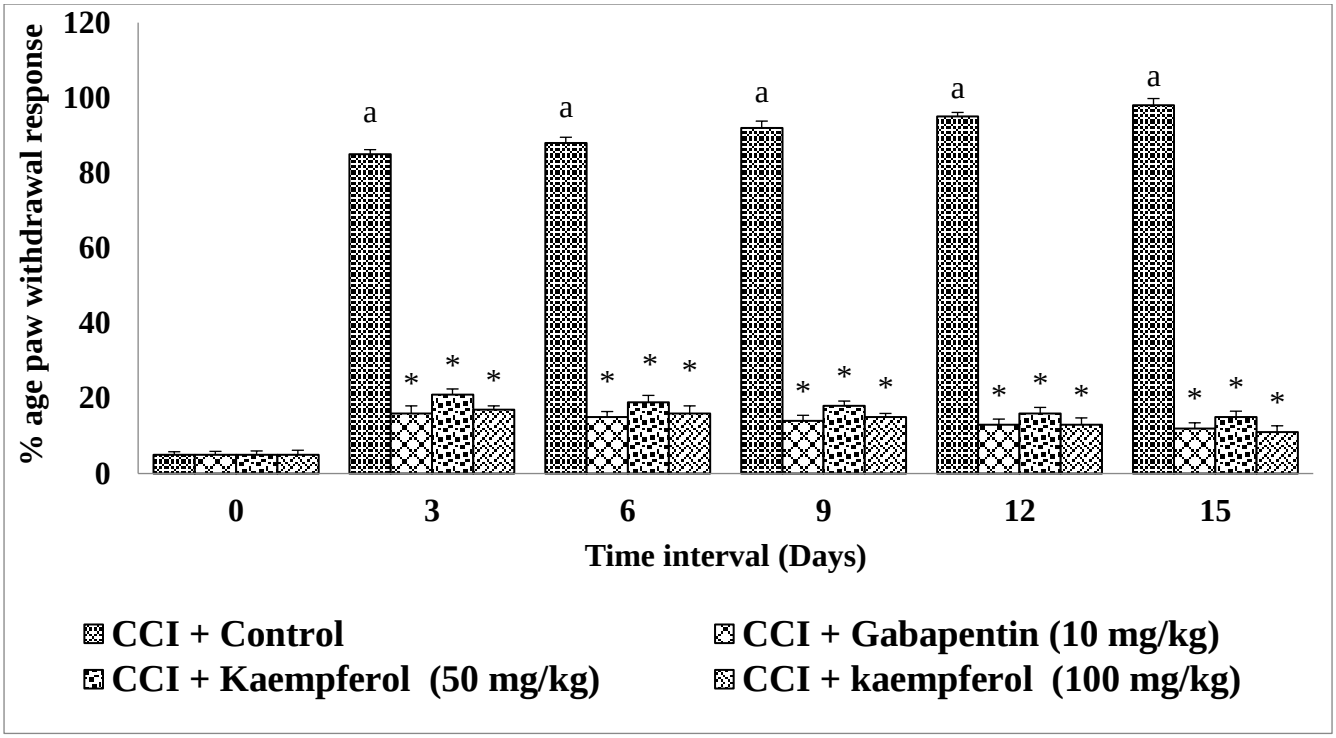

Figure 1: Neuroprotective profile of CCI induced neuropathic pain of kaempferol using Von Frey hair filament test.

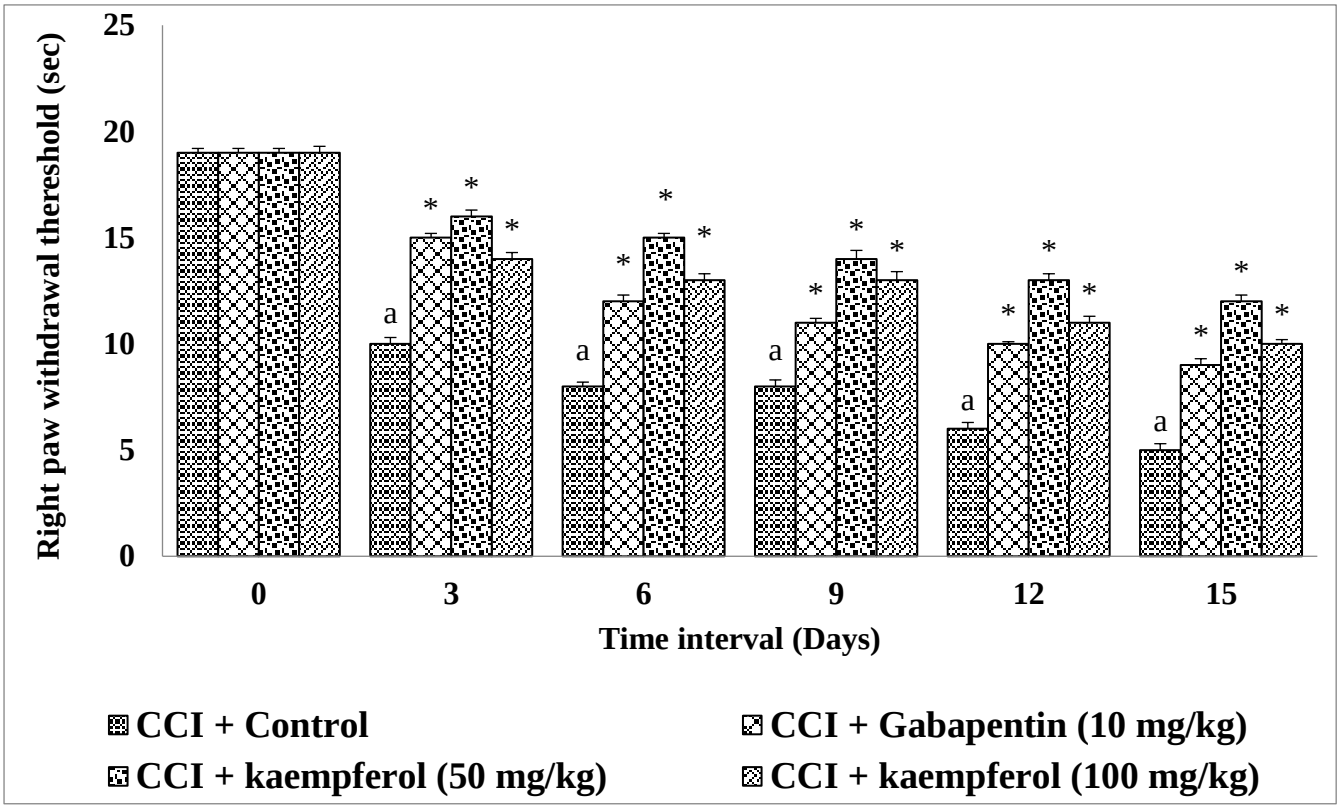

Figure 2: Neuroprotective profile of CCI induced neuropathic pain of kaempferol using Hargreaves test.

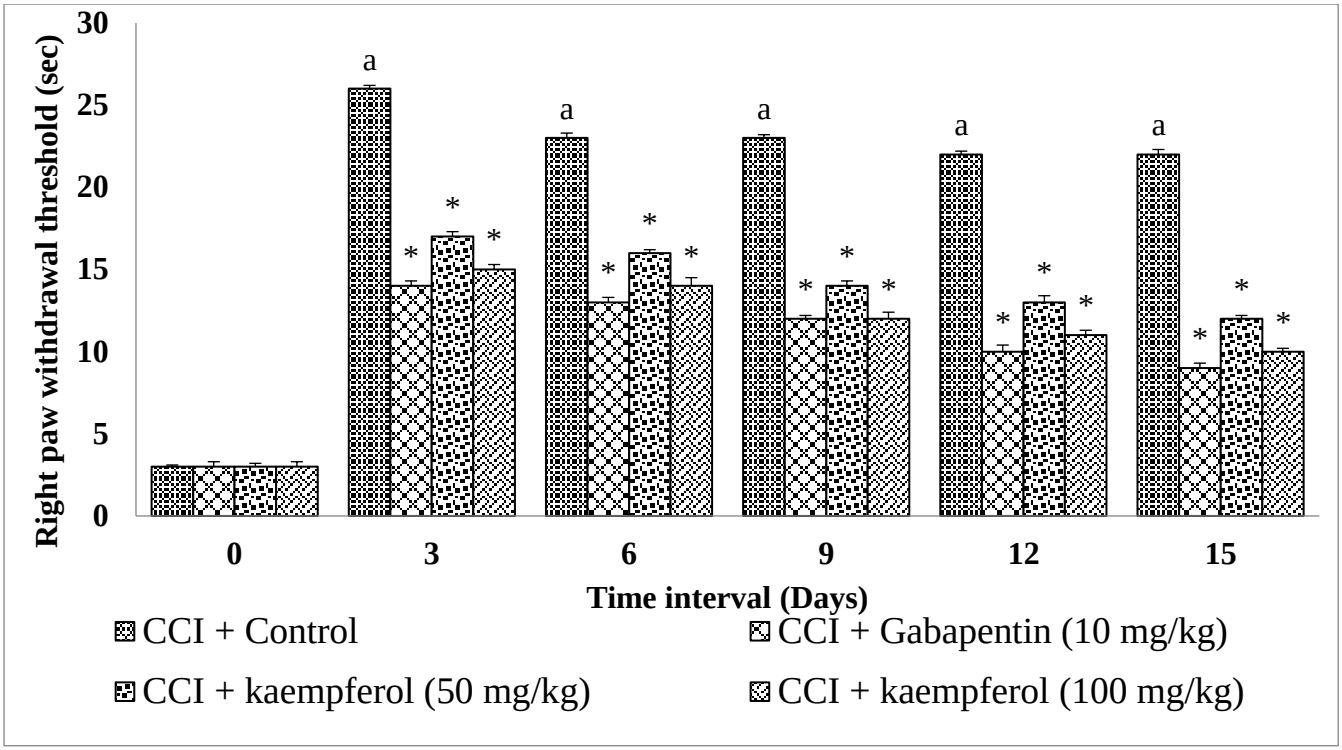

Figure 3: Neuroprotective profile of CCI induced neuropathic pain of kaempferol using pin prick test.

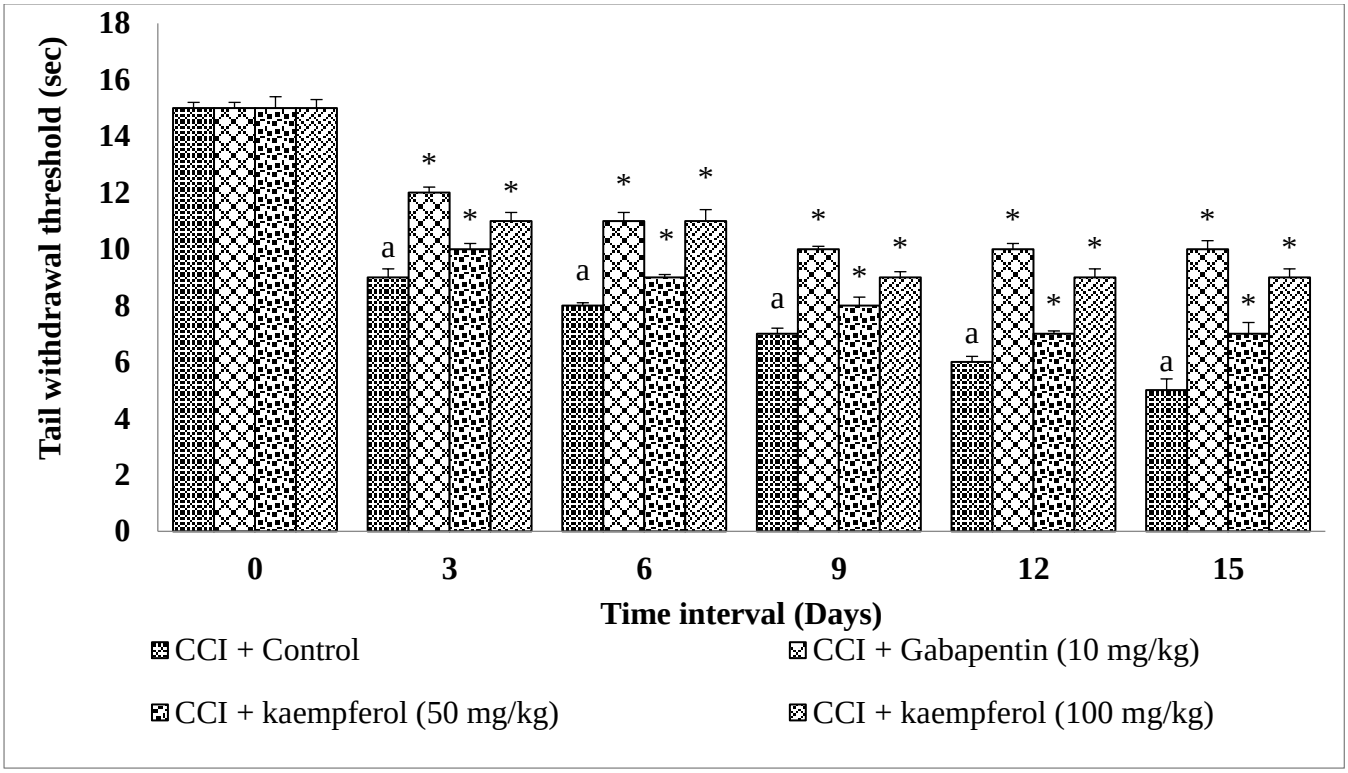

Figure 4: Neuroprotective profile of CCI induced neuropathic pain of kaempferol using D'Aemour and Smith test.

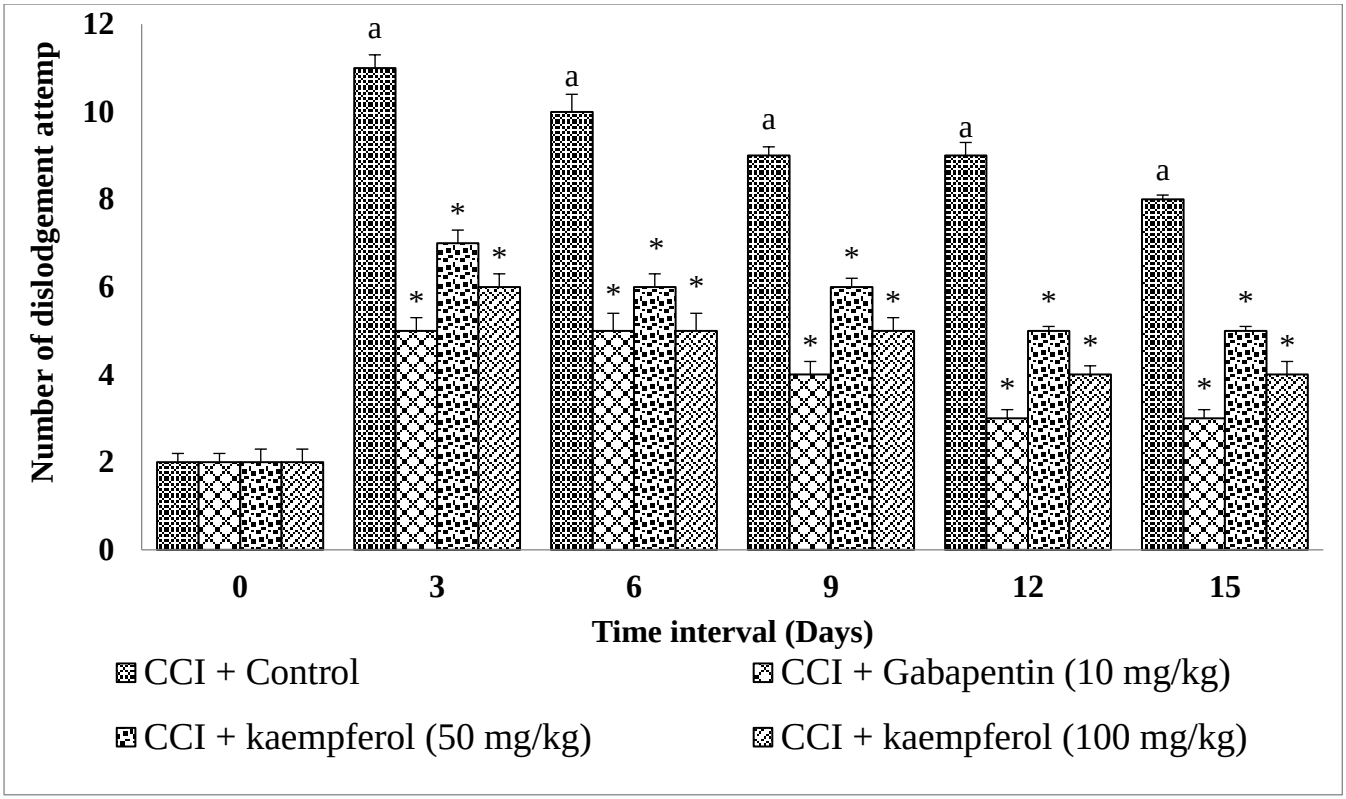

Figure 5: Neuroprotective profile of CCI induced neuropathic pain of kaempferol using tail pinch test.

Effect of kaempferol on CCI induced tissue biomarker changes

TBARS, GSH, and total proteins were quantitatively determined using standard curves for TMP (figure 6), GSH (figure 7), and BSA (figure 8). Table 1 clearly shows that the higher level of TBARS in neuropathic pain is significantly reduced from the control and statistically equivalent to gabapentin after treatment with kaempferol (50 or 100 mg/kg, p.o.). In contrast, the decreased level of GSH in neuropathic pain is significantly increased from the control and statistically equivalent to gabapentin after treatment with kaempferol (50 or 100 mg/kg, p.o.). Following therapy with kaempferol (50 or 100 mg/kg, p.o.), the lowered con tent of total protein in neuropathic pain increases in comparison to control and is statistically equal to gabapentin.

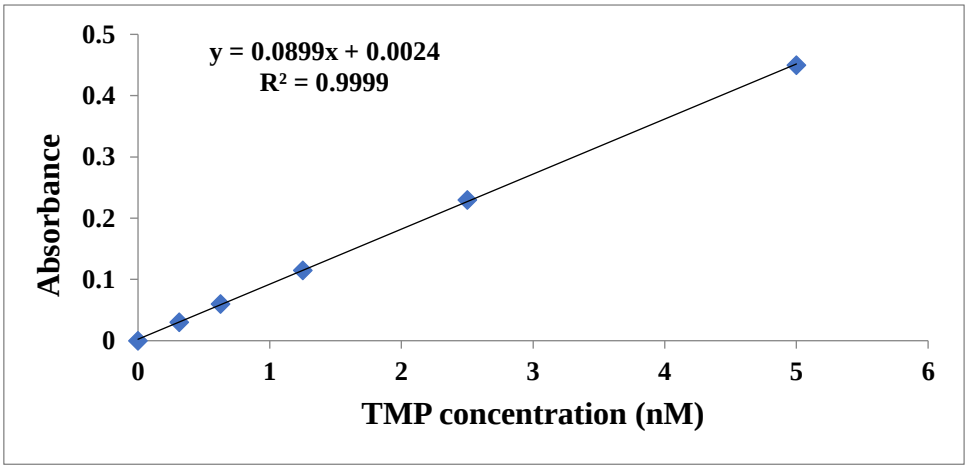

Figure 6: Standard plot of TMP vs absorbance.

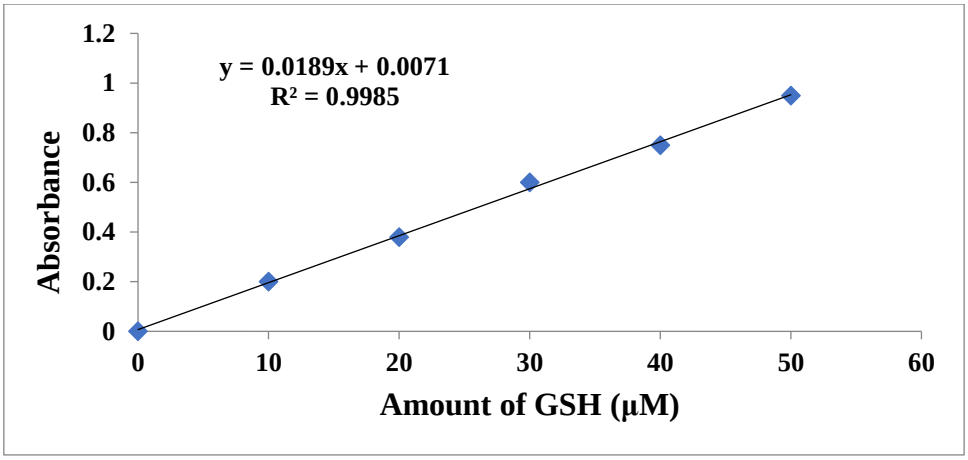

Figure 7: Standard plot of GSH vs absorbance.

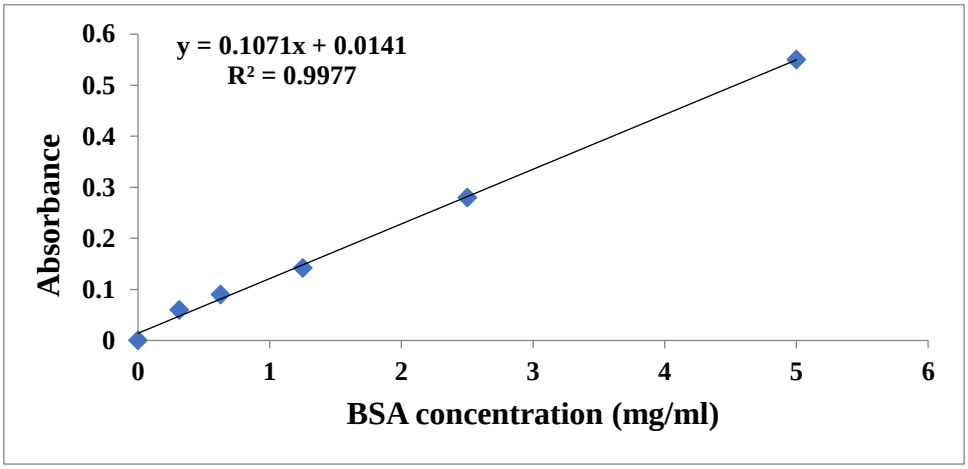

Figure 8: Standard plot of BSA vs absorbance.

Table 1: Effect of kaempferol on CCI induced oxidative stress marker changes.

Groups	**TBARS** (nM / mg of protein)	**GSH** (µM / mg of protein)	**Total proteins** (mg / ml)
CCI + Control	13.45 ± 0.90[a]	30.25 ± 2.10[a]	51.25 ± 3.25[a]
CCI + Gabapentin (10 mg/kg)	4.90 ± 0.58[*]	68.40 ± 5.25*	103.80 ± 6.89[*]
CCI + kaempferol (50 mg/kg)	5.80 ± 1.12[*]	57.25 ± 5.36[*]	89.12 ± 7.80[*]
CCI + kaempferol (100 mg/kg)	5.10 ± 0.60[*]	63.40 ± 6.80[*]	95.70 ± 5.88****

In the present study, CCI of sciatic nerve in rat has produced a major increase in thermal hyeralgesia, thermal allodynia, mechanical hyperalgesia and mechanical allodynia in peripheral and central site with alteration of various biochemical parameters *i.e.*, rise in TBARS and decrease in reduced glutathione levels. Administration of kaempferol (50 to 100 mg/Kg; *p.o.*) more overly reduces the CCI of sciatic nerve induced above behavioral and biochemical changes.

Rats with mononeuritic neuropathic pain are frequently treated with the sciatic nerve model's CCI. It clinically resembles musculoskeletal conditions associated with the workplace, including tarsal tunnel syndrome and carpal tunnel syndrome. Complex regional pain syndrome (CRPS) is another name for it. The pathophysiology of neuropathic pain in humans and experimental animals has been found to involve a number of pathogenic mechanisms and

targets. The main causes of neuropathic pain brought on by peripheral nerve injury are the production of free radicals, cytokines, and changes in ionic motions. It is generally known that CCI-induced neuropathic pain is caused by the buildup of calcium, tumor necrosis factor, and free radicals in the sciatic nerve. Furthermore, the activation of lipid peroxidation in the neurological system is also caused by an abundance of free radicals. These alterations are the cause of the ischemia environment in the peripheral nervous system, which intensifies peripheral neurodegeneration. Central neuropathic pain is subsequently caused by peripheral nerve injury, which also modifies the GABAnergic signaling pathways in the brain (Dhir et al., 2020; Bharti et al., 2021; Hussain et al., 2022).

By preventing the deposition of amyloid fibrils (like Aβ, tau, and α-synuclein), inhibiting microglia activation, lowering the release of inflammatory factors, restoring the mitochondrial membrane to prevent oxidative stress, shielding the blood-brain barrier, and blocking certain enzyme activities (like cholinesterase), kaempferol and its derivatives primarily provide neuroprotection. Natural neuroprotective agents that show promise include kaempferol and its derivatives. Kaempferol and its derivatives may be novel therapeutic candidates for the treatment of neurological disorders if their pharmacological mechanism is understood (Jin et al., 2023).

CONCLUSION

Hence, kaempferol is produce the ameliorative effect in CCI induced neuropathic pain via reduction of free radical generation, lipid peroxidative action.

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