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Therapeutic Potential of Rutin in the Management of Chronic Constriction Injury of Sciatic Nerve Induced Neuropathic Pain in Rats

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ABSTRACT

The present study was designed to investigate the role of rutin 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 batteries of behavioural 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) and reduced glutathione (GSH) were also estimated in sciatic nerve tissue sample. The administration of rutin (25 and 50 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, rutin may be a useful medicine for the management of neuropathic pain.

Keywords: Allodynia, Chronic constriction injury, Neuropathic pain, Sciatic nerve, Rutin.

INTRODUCTION

Traditionally neuropathic pain is considered as nociceptive pain disorders. The epidemiology of neuropathic pain revealed that 1 % and 2 % neuropathic pain patients from overall world population are present in the United Kingdom[1] and United States of America, respectively[2]. The world-wide diabetic neuropathic pain was noted 2.8% in 2000 and second most cases of neuropathic pain is due to herpes zoster infection which is observed in 24% at 2004[3]. In addition, 37.0% neuropathic pain was observed with chronic lower back pain[4]. The older age above 70 are more prone to develop the neuralgia as compared to below 60 years old, the difference is observed almost 3% of postherpetic neuralgia patients[5]. Recent report suggested that, prevalence of neuropathic pain is 1-2% lower than that of classic symptoms rate 6-8% after treatment[2]. The females are superior to male species for development of neuropathic pain due to the hormonal variability[6]. Neuropathic pain has been cured with aid of various allopathic medications morphine, oxycodone, tramadol, codeine and dihydrocodeine[7]. Whereas, these agents has been reported to produce serious toxic reactions due to chronic administration schedule in neuropathic patients[8]. Currently various herbal approach proved that, phytomedicines such as curcumin, berberine and vitamin E are play a promising role in the amelioration of neurodegeneration and neuropathic pain[9].

Rutin is a bioflavonoid available in several medicinal plants[10]. It has potent neuroprotective action on prion peptide-induced dopaminergic neuronal cells by inhibiting apoptotic pathway[11]. The rutin has been reported to produce antioxidant, anti-apoptotic, reduction of p53 expression, regulation of TNF-α & Bcl-2 protein expressions[12], antianxiety[13], antiamnesic[14], antistress[15] and anticonvulsant. However, the role of rutin in entrapment neuropathy has not been studied so far. Therefore, this protocol is designed to explore role of rutin in the amelioration of chronic constriction injury of sciatic nerve induced neuropathic pain in rat.

MATERIALS AND METHODS

Animals

Male Sprague Dawley rat of 200-250 g were procured from Sanjay Biological, Amritsar, Punjab, India. The present protocol was sanction by Institutional Animal Ethics Committee (IAEC No.: ATRC/01/15; Dated: 19/12/15).

Induction of Peripheral Neuropathic Pain

Neuropathic pain was induced in rats by standard protocol reported in scientific literature such as chronic constriction injury (CCI) of sciatic nerve[16].

Behavioral Evaluation

Evaluation of behavioral parameters was carried out at different time span *i.e.,* 0, 4, 8, 12 and 16th day 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 experimental models such as Von Frey hair filament test[17], Hargreaves test[18], pin prick test[19], D'Aemour and Smith test[20] and tail pinch test[21].

Biochemical Analysis

All groups of animals were sacrificed after 16th day of behavioural observation by cervical dislocation and complete right sciatic nerves were isolated immediately. 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)[22] and reduced glutathione (GSH)[23].

Experimental Protocol

In the present study, eight groups were introduced; each consists of six rats. All the behavioral and biochemical evaluations were carried out as described in group I for groups II-VIII. Group I (Normal control): In this group rats were subjected to study of neuropathic pain sensitivity without involvement of any surgical procedure and drug administration. All six animals were under observation for 16 successive days. After the behavioural assessment all the animals were sacrificed on 16th day for the presence of TBARS and (GSH). Group II (Sham control): Rats were allowed to pursue for a surgical procedure to expose right sciatic nerve without any nerve ligation in order to distinguish the pain originating from the CCI of sciatic nerve group. Group III (CCI group): Neuropathic pain by CCI of sciatic nerve was induced in all the rats. The detailed elaboration regarding the technique was mentioned in preliminary section. Group IV & V (Rutin per se & gabapentin per se): For 16 consecutive days, rats were administered rutin (50 mg/kg, *p.o.*) and gabapentin (10 mg/kg, *p.o.*) respectively. Group VI & VII (Rutin therapy): Rutin (25 and 50 mg/kg, *p.o.*) was provided for 16 consecutive days to all the rats. Group VIII (Gabapentin therapy): Rats were allowed for gabapentin (10 mg/kg, *p.o.*) treatment for 16 consecutive days.

Statistical Analysis

Data were expressed as mean±SD, n=6 rat per group. $^{a}P<0.05$ Vs sham control group; $^{b}P<0.05$ Vs CCI control group; $^{c}P<0.05$ Vs gabapentin treated group. The results were analyzed statistically using two-way analysis of variance (ANOVA) followed by Bonferonni's *post-hoc* analysis using Graph pad prism Version-5.0 software.

RESULTS AND DISCUSSION

Peripheral mechanical allodynia was developed in the CCI of sciatic nerve, indicated by elevation in the percentage withdrawal latency of the paw as comparison to sham control group. Treatment of rutin (25 and 50 mg/kg, for 16 consecutive days) produces a remarkable response in CCI rising of peripheral mechanical nociceptive pain sensation and statistically equal to gabapentin. Further, rutin *per se* and gabapentin *per se* treated groups did not show any notable changes of CCI induced mechanical allodynia in rat (Fig. 1).

Decrease in right hind paw withdrawal threshold in CCI of sciatic nerve indicates the development of thermal hyperalgesia when compared to sham group. The dose of 25 and 50 mg/kg of rutin, for 16 consecutive days significantly attenuate the CCI induced decrease in the thermal nociceptive pain threshold and statistically equal to gabapentin. Moreover, rutin *per se* and gabapentin *per se* treated group did not express any significant changes of CCI induced thermal hyperalgesia in rat (Fig. 2).

The development of peripheral mechanical hyperalgesia in the CCI of sciatic nerve causes a desirable raising of the percentage paw withdrawal response in contrast to sham control group. Raising of peripheral mechanical nociceptive pain sensation due to CCI was highly marked with the dose of 25 and 50 mg/kg of rutin for 16 consecutive days and statistically equal to gabapentin. However, rutin *per se* and gabapentin *per se* treated groups did not demonstrate changes in the CCI induced mechanical hyperalgesia in rat (Fig. 3).

Progress in the thermal hyperalgesia due to CCI of sciatic nerve was marked by decrease in tail withdrawal threshold as comparison to sham group. The dose of 25 and 50 mg/kg of rutin, significantly attenuated CCI induced reduction in the thermal nociceptive pain threshold and statistically equal to gabapentin. However, rutin *per se* and gabapentin *per se* treated group did not show any significant changes of CCI induced thermal hyperalgesia in rat (Fig. 4).

Outcome of central mechanical hyperalgesia in CCI of sciatic nerve was demonstrated by elevation in the number of dislodgements attempt as compare to sham group. Further, 25 and 50 mg/kg, of rutin treatment attenuated CCI caused raise in the central mechanical nociceptive pain sensation in a dose dependent manner. Gabapentin also induces identical effects. However, rutin *per se* and gabapentin *per se* treated group did not cause any remarkable alteration in CCI induced mechanical hyperalgesia (Fig. 5).

CCI of sciatic nerve resulted in a remarkable elevation in TBARS; drop in reduced glutathione content as comparison to sham group. Administration of rutin (25 and 50 mg/kg) significantly attenuate CCI induced changes of tissue biomarkers in a dose dependent manner in compassion to standard group. However, rutin *per se* and gabapentin *per se* treated groups did not express notable changes in CCI induced tissue biomarker changes in rat (Table 1).

The CCI of sciatic nerve model is very commonly employed for the development mononeuritic neuropathic pain in rat[16]. It is clinically mimics the work-related musculoskeletal disorders such as carpal and tarsal tunnel syndrome[24]. The generation of free radicals, cytokines and alteration of ionic movements are involved as primary changes in the peripheral nerve injury induced neuropathic pain and lipid peroxidation in the nervous system. The accumulation of free radical, tumour necrosis factor and calcium in sciatic nerve is well established in CCI induced neuropathic pain[25,26]. These changes are responsible

to produce the ischemic environment in the peripheral nervous system and it undergoes the enhancement of neurodegeneration at peripheral site[27]. Subsequently, peripheral nerve injury also alters the GABAnergic signaling processes in brain leads to produce the central neuropathic pain[28].

Rutin has great potential to scavenge the free radicals and it also produces the regulatory effects of vascular integrity[14]. In addition, the molecular mechanism of rutin had shown that, it has regulatory role in oxidative stress, glucose uptake, activation of mitogen-activated protein kinases (MAPK) and nuclear factor kappa-activated B cells (NF-κB) pathway[29], including the regulation of ATPases[30]. These factors are also contributed to development of painful neuropathy. In this study, rutin plays a role in the amelioration of CCI of sciatic nerve induced peripheral neuropathic pain along with reduction tissue oxidative stress marker. Gapapentin is one of the anti-convulsant agents and is reported to relieve the pain associated with neuropathy in laboratory rodents and in human being[31]. Furthermore, rutin has neuroprotective action via allosteric modulation of $GABA_A$ receptor by interaction of benzodiazepine binding site[14]. Based on this study and other literature, rutin has multi-targeted therapeutic potential in various neurological diseases[14]. Hence, it produces the neuroprotective action in various neurological diseases such as stress induced anxiety[13], cerebral hypoperfusion induced vascular dementia and Alzheimer's disease[14] and cerebral ischemic disease like stroke[15]. In agreement to the present studies, it can be concluded that various flavonoids are used to treat neuropathic pain such as chrysin[32], naringenin[33] and p-coumaric acid[34].

CONCLUSION

Finally, it can be concluded that rutin is produce the ameliorative effect in CCI induced neuropathic pain via reduction of free radical generation and lipid peroxidative action.

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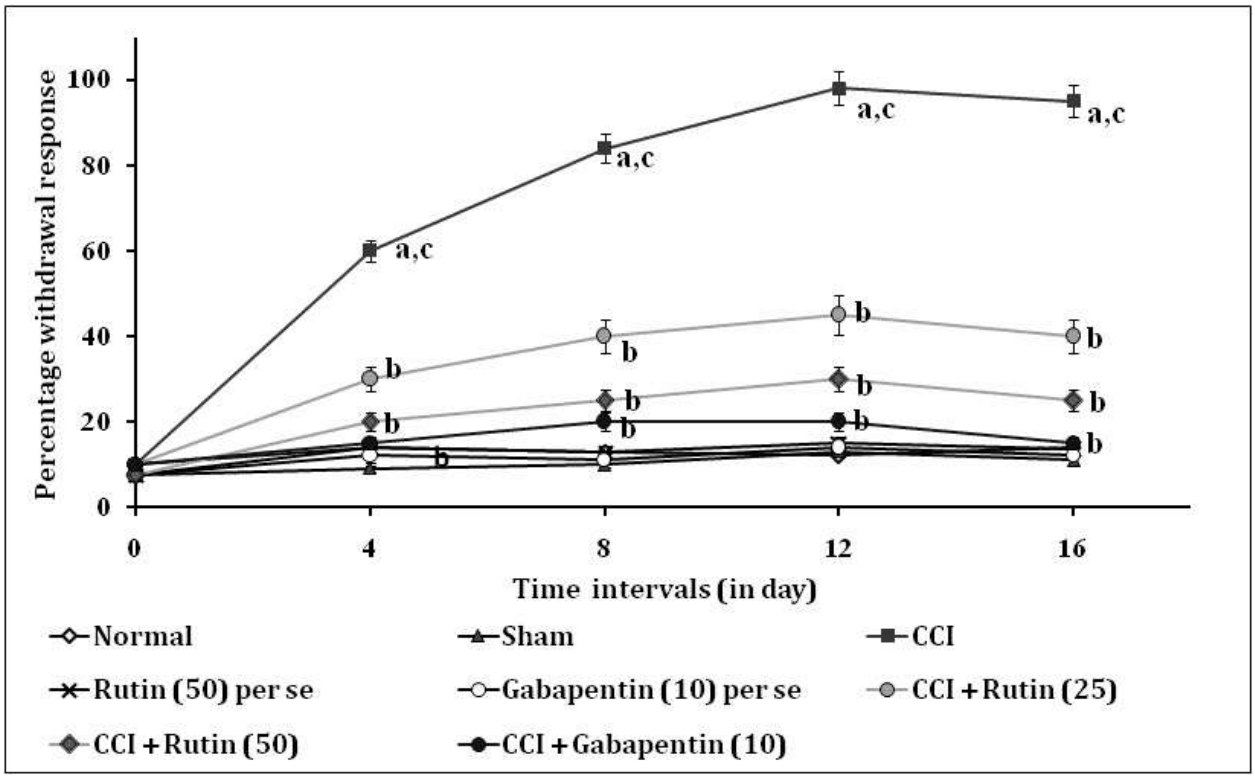

Fig.1: Effect of rutin on Von Frey hair filament test

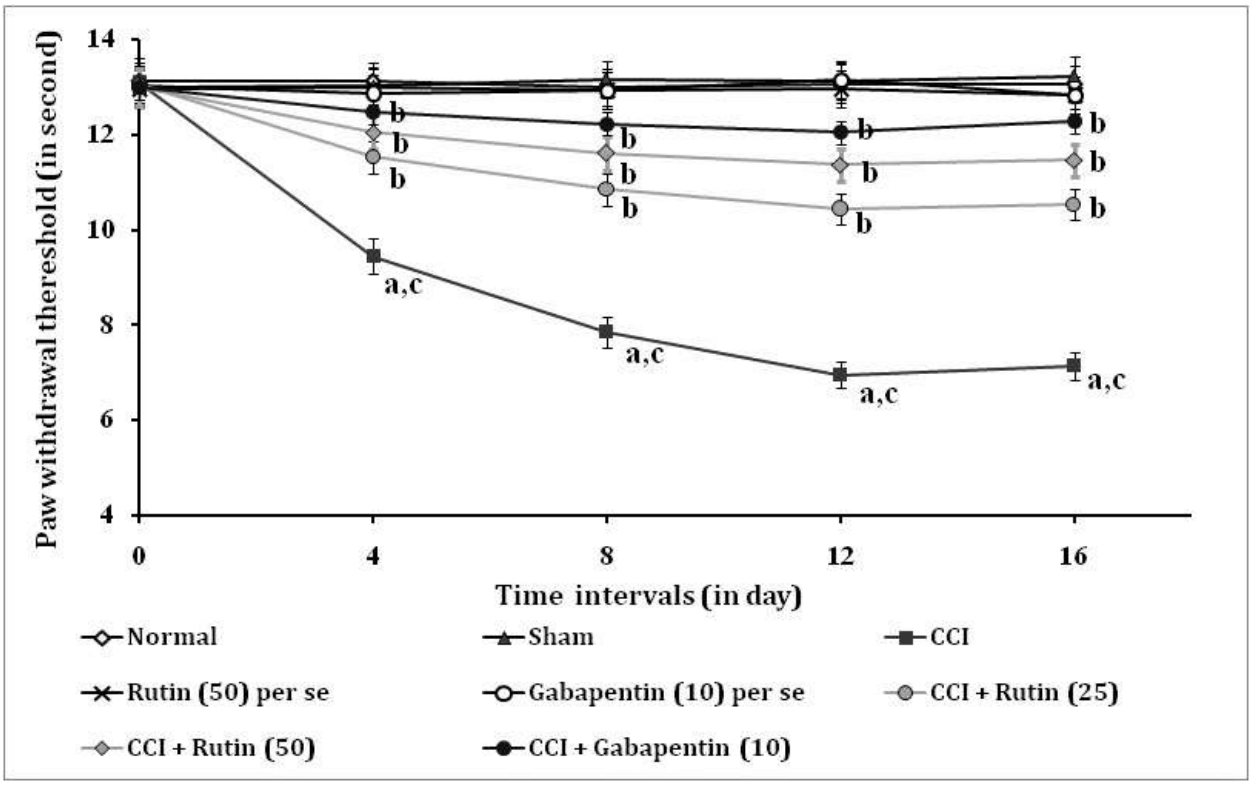

Fig. 2: Effect of rutin on Hargreaves test

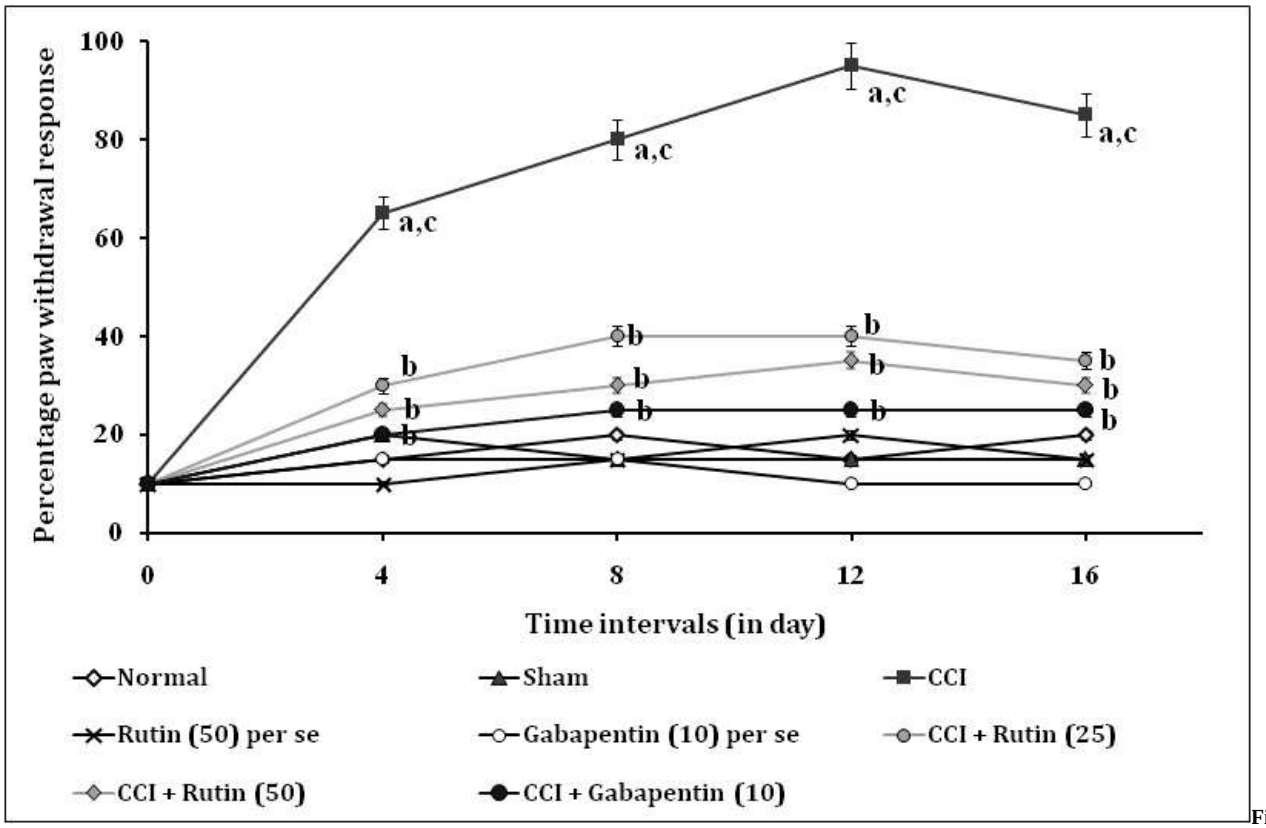

Fig. 3: Effect of rutin on pin prick test

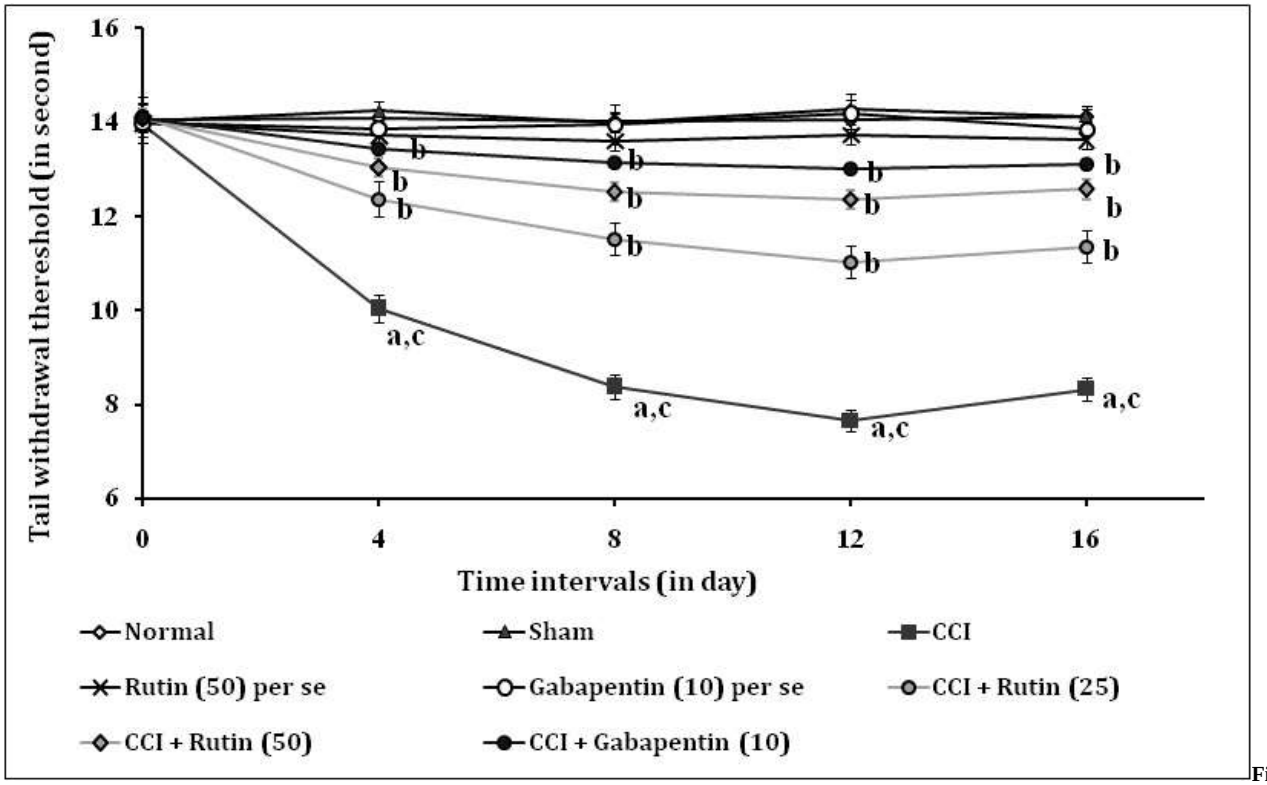

Fig. 4: Effect of rutin on D'Amour and Smith test

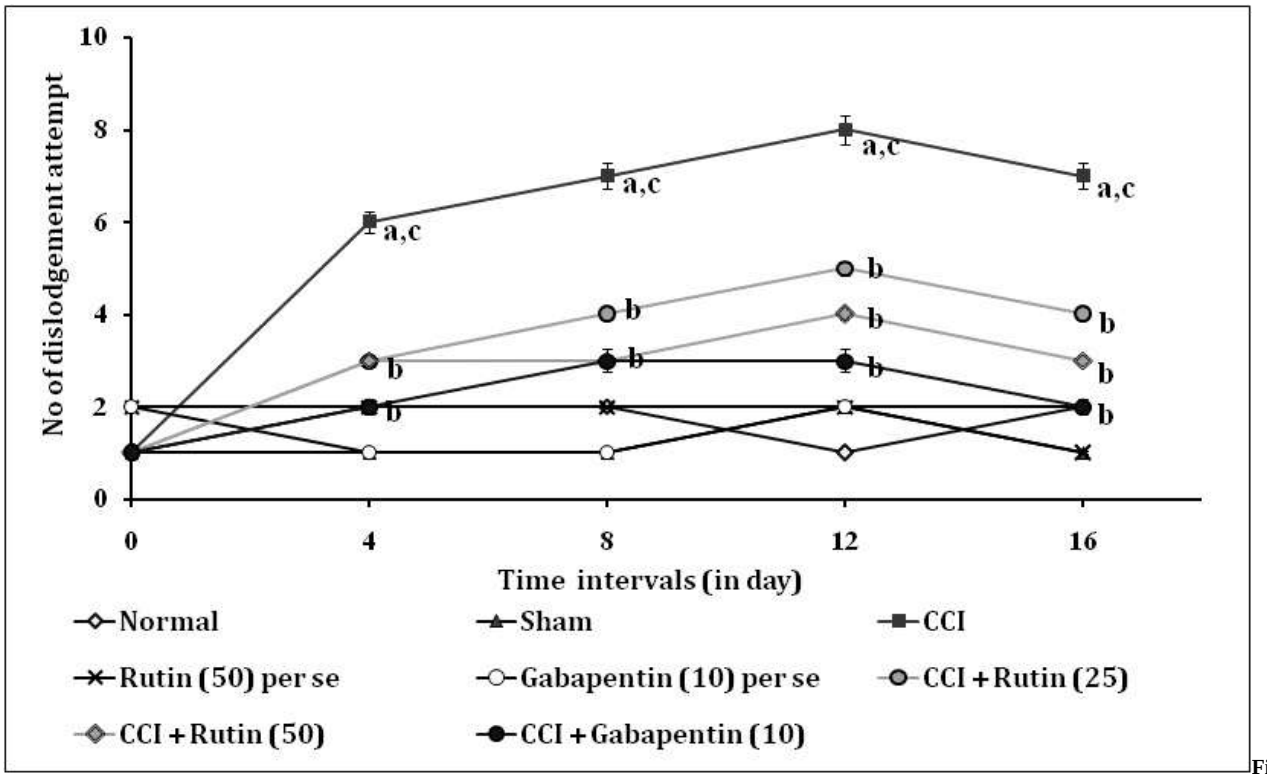

Fig. 5: Effect of rutin on tail pinch test

Table 1: Effect of rutin on cci induced oxidative stress marker changes

Groups	**TBARS (nM / mg of protein)**	**GSH (µM / mg of protein)**
Normal	2.96 ± 0.72	75.42 ± 2.82
Sham	2.98 ± 0.91	77.02 ± 2.91
CCI	9.04 ± 1.03[a]	32.79 ± 3.041[a]
Rutin (50) *per se*	2.89 ± 0.99	74.48 ± 2.21
Gabapentin (10) *per se*	3.31 ± 0.74	73.84 ± 2.73
CCI + Rutin (25)	4.72 ± 1.03[b]	66.94 ± 1.94[b]
CCI + Rutin (50)	4.05 ± 0.86[b]	69.52 ± 2.63[b]
CCI + Gabapentin (10)	3.32 ± 0.97[b]	72.63 ± 2.59[b]