

## Pentoxifylline decreases allodynia and hyperalgesia in a rat model of neuropathic pain

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### ABSTRACT

*Background and the purpose of the study:* Pentoxifylline (PTX) is a non-specific cytokine inhibitor that has been reported to attenuate pain in several animal models and humans. However, long-term therapeutic effects of PTX on neuropathic pain in a rat model of chronic constriction injury (CCI) are not completely clear. This study was conducted to examine the effect of long-term administration of PTX on neuropathic pain in rats.

*Methods:* Neuropathic pain was induced by sciatic nerve ligation using of CCI model in rats. Rats were randomly assigned into sham, CCI-saline treated, and CCI-PTX treated (30 or 60 mg/kg ip) groups. PTX or saline administered at 30 min before CCI and daily for 14 days post-CCI. At the days of 3, 7, 11 and 14 following CCI, by using standard methods effects of thermal hyperalgesia, thermal and mechanical allodynia in all groups were examined using the standard methods.

*Results:* The CCI-saline treated group showed a significant increase in mechanical and thermal allodynia, and thermal hyperalgesia as compared with the sham group in the tested days. Administration of the higher dose of PTX (60 mg/kg/day), but not the lower dose (30 mg/kg/day) significantly reduced mechanical and thermal allodynia, as compared with the CCI-saline treated group on days of 3, 7, 11 and 14 (all P values < 0.001). Also, both doses of PTX significantly reduced thermal hyperalgesia as compared with the CCI-saline treated group on these days (all P values < 0.001).

*Conclusion:* Results of this study show that chronic administration of PTX reduces the neuropathic pain in a rat model of CCI. Thus, this drug may have a therapeutic application in the treatment and management of neuropathic pain in humans.

**Keywords:** Chronic Constriction Injury (CCI), Thermal hyperalgesia, Thermal and mechanical allodynia.

### INTRODUCTION

Neuropathic pain is a persistent pain which induced following peripheral or central nervous system injuries (1). Hyperalgesia (an increased response to normally painful stimuli) and allodynia (a painful response to normally harmless stimuli) are abnormal sensory signs which are usually observed along neuropathic pain (1). These behavioral parameters have been often used to study the pharmacology and modulation of neuropathic pain (1). In spite of many investigations the underlying mechanisms of with neuropathic pain and its pathophysiology are not completely clear (1-5).

Recently several evidences suggested that pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF- $\alpha$ ) and interleukin-1 might be involved in the genesis, persistence and severity of neuropathic pain (1, 6-8). It has been demonstrated

that administration of anti-TNF-alpha and IL-1b monoclonal antibodies provided analgesia in rheumatoid arthritis patients (9). Nowadays, pro-inflammatory cytokines is a new approach for the management of inflammatory and neuropathic pain in clinical situations.

Pentoxifylline (PTX), an inhibitor of phosphodiesterase and non-specific inhibitor of cytokine (10, 11), which inhibits the synthesis of TNF- $\alpha$ , interleukin-1 (IL-1), IL-6, and IL-8 (12-14). Previous studies have shown beneficial effects of PTX in dermatitis, leprosy, rheumatoid arthritis, cancer (15-17) and cerebral ischemia (18, 19). A few studies have demonstrated that PTX is able to attenuate the formalin-induced pain behavior in rats (12) and post-operative pain in patients (20). Additionally, the anti-hyperalgesic effects of PTX in three experimental inflammatory pain models

have been demonstrated and it is shown that this effect was associated with inhibition of TNF- $\alpha$  synthesis (7). Study of Liu and his colleagues showed that PTX significantly decreased mechanical and thermal hyperalgesia in rats with L5 nerve transection model (13). A recent study showed PTX decreased allodynia and hyperalgesia considerably on the day of seven after CCI in rat and mice (21).

Taken together, long-term therapeutic effects of PTX on neuropathic pain in rat model of CCI is not completely clear. Therefore, the aim of this study was to examine whether pre-emptive and repeated systemic administration of PTX for two weeks could attenuate mechanical and thermal allodynia and hyperalgesia in rat model of neuropathic pain.

## MATERIAL AND METHODS

### *Animals and Drug*

Adult male Wistar rats (200-250 g) were obtained from breeding colony of Semnan University of Medical Sciences (SUMS), Semnan, Iran. All rats were housed in cages in a 12-h light/dark cycle at 22–24°C, with food and water ad libitum. All procedures were conducted in agreement with the National Institutes of Health Guide for Care and Use of Laboratory Animals.

PTX was purchased from Fluka company (Germany). The drug was dissolved in physiological saline and injected at doses of 30 or 60 mg/kg (i.p). These doses were selected on the basis of the previous pilot studies (14, 18).

### *Neuropathic pain model*

Chronic constriction injury model was used to induce neuropathic pain as it was previously described (22). Briefly, under ketamine/xylazine (80 mg/kg and 10 mg/kg, i.p respectively) anesthesia the left sciatic nerve was exposed and then four loss ligatures (4/0 cutgoat plain) was placed around the nerve with 1mm spacing until a brief twitch in the hind limb was observed. Afterward, in all animal's thermal hyperalgesia, mechanical and thermal allodynia were evaluated on days of 3, 7, 11 and 14 after surgery or CCI.

### *Experimental protocol*

Forty rat were assigned randomly to four groups (each, n=10). Group 1 was sham-operated (surgery without induction of CCI). Group 2 was the control, which received saline (1 ml/kg). Groups 3 and 4 were treated with PTX at doses of 30 mg/kg and 60 mg/kg, respectively. In all groups, PTX or saline was given 30 min before CCI and then daily for 14 days post-CCI. Next, behavioral tests including thermal hyperalgesia, mechanical and

thermal allodynia were performed in each group on days of 3, 7, 11 and 14 after surgery or CCI (see below).

### *Behavioral tests*

#### *Mechanical allodynia (Von Frey test)*

Mechanical allodynia was measured using a series of von Frey filaments (Stoelting, Wood Dale, IL, USA), ranging from 2 to 60 g (23). To measure mechanical allodynia, each rat was placed in plastic cages with wire net floor, were allowed to move freely. All animals were allowed to adapt to this environment for 5 min before testing. Afterward, the Von Frey filament was applied by gentle increase in the strength (2-60 g) to left dorsal surface of the hind paw until the rat lifted the paw away (21, 23). Three tests were performed at intervals of 5 min and the applied force (g) was recorded.

#### *Thermal hyperalgesia*

Thermal hyperalgesia was determined by using paw withdrawal latencies to radiant heat (Model 390, IITC Life Science, INC.). After adaption of animals to the Plexiglas cage, the movable radiant heat source under the glass floor was focused on the plantar surface of the hind paw until animal lifted the paw away. The paw withdrawal latencies were measured for left and right hind paw of each animal for three times with intervals of 5 min and a cut-off time was set at 20 s to avoid tissue damage.

#### *Thermal allodynia*

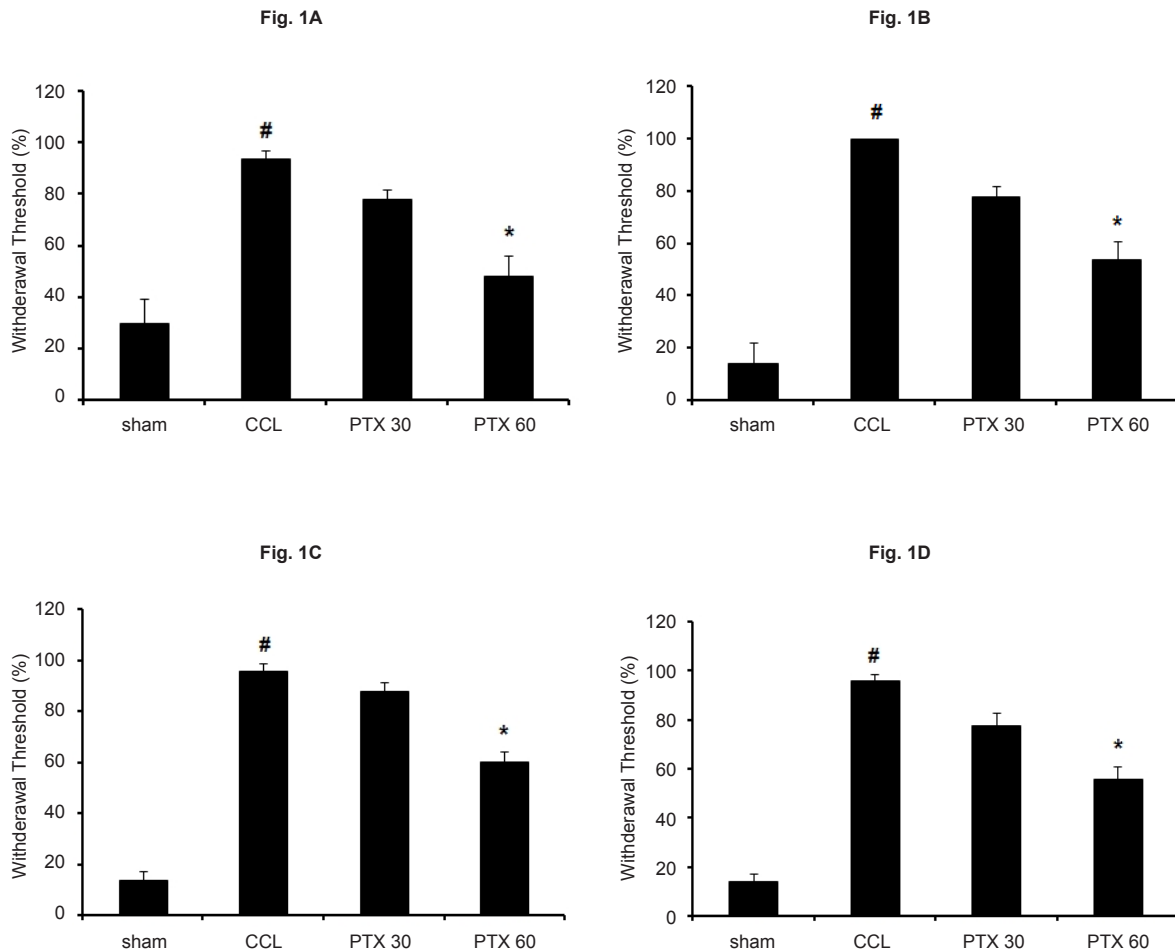
Acetone test was used to determine thermal allodynia. Rats were placed in plastic cages with a wire-mesh floor and after adaptation to environment by using of syringe filled with acetone, animals were pushed to left hind paw until the animal lifted the paw away. Three tests were done 5 times at intervals of 3 min. Withdraw hind paw was considered as a positive response.

#### *Statistical analyses*

Data are presented as mean  $\pm$  SEM. Thermal and mechanical allodynia data were analyzed by non-parametric Kruskal-Wallis ANOVA on ranks followed by a Dunn's test (SigmaStat 2.0, Jandel Scientific, Erkrath, Germany). Thermal hyperalgesia was analyzed by one way analysis of variance (ANOVA) followed by Dunnet test as post hoc analysis. Differences were considered significant at  $P < 0.05$ .

## RESULTS

Administration of PTX (60 mg/kg) 30 min before CCI and then once a day for 14 days, significantly reduced the percent of withdrawal threshold (thermal allodynia) and mechanical threshold (mechanical allodynia) on days of 3, 7, 11 and 14 after CCI as compared with saline-treated CCI group (Figs. 1, 2A,



**Figure 1.** Effects of pre-emptive and repeated administration of pentoxifylline (PTX) at doses of 30 mg/kg (PTX-30) and 60 mg/kg (PTX-60) on the percent of withdrawal threshold (thermal allodynia, Acetone test) on days of 3(A), 7(B), 11(C) and 14(D) after injury in rat. The data are presented as the Mean $\pm$ SEM. # $P$ <0.001 versus Sham group; \* $P$ <0.001 versus saline-treated chronic constriction injury (CCI).

B, C and D;  $P$ <0.001). The lower dose (30 mg/kg), of PTX was effective on these measures except on the day of 3 after injury (Figs. 1, 2A, B, C and D;  $P$ >0.05).

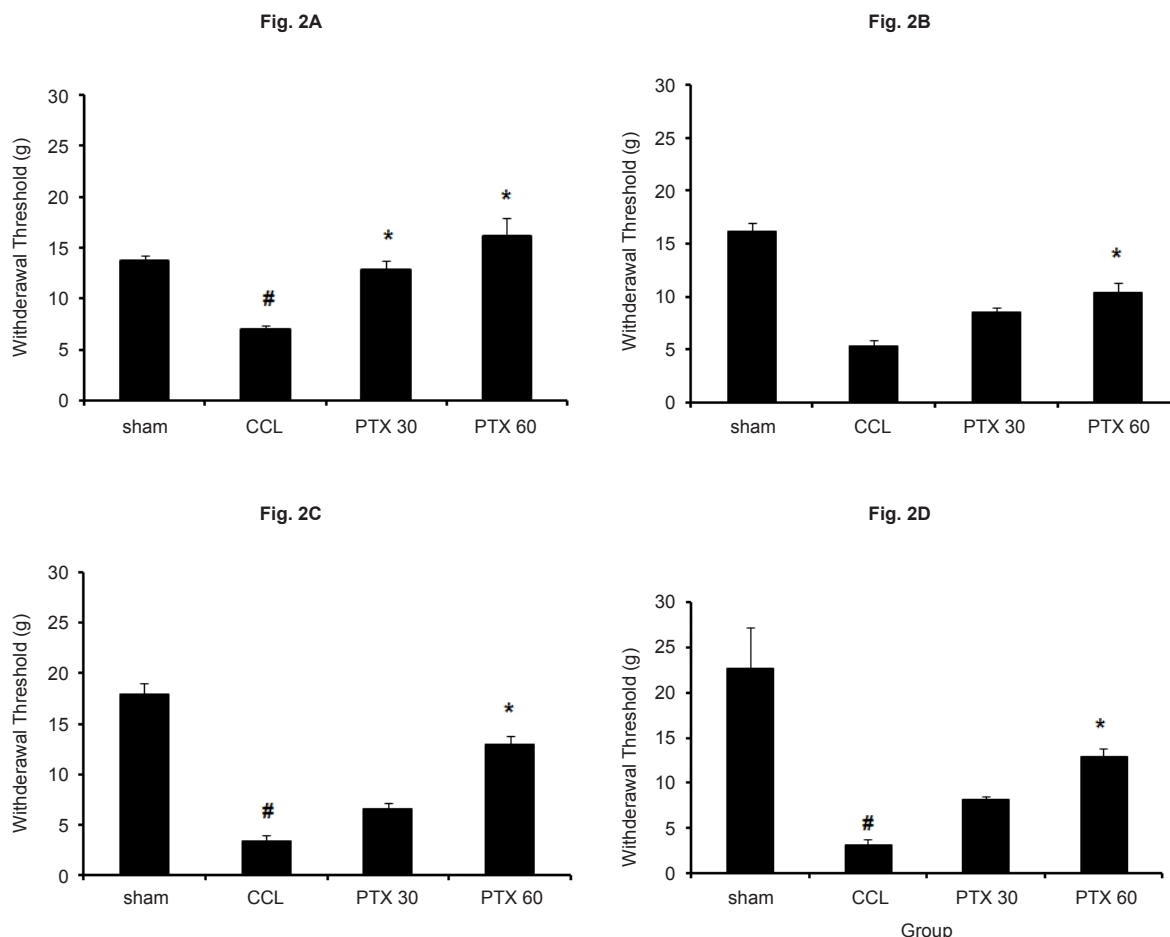
PTX at both doses (30 and 60 mg/kg) significantly reduced thermal threshold (thermal hyperalgesia) in all days after CCI as compared with control group (Fig. 3A, B, C and D;  $P$ <0.001).

#### DISCUSSION

In this study, a rat model of CCI was used to study chronic pain because it mimics clinical condition of chronic nerve compression such as spinal root irritation by a lumbar disk herniation in humans (1). Strong mechanical, thermal allodynia and thermal hyperalgesia appeared from the third day after injury, which is consistent with results of other studies (21, 23).

It was found that chronic application of PTX only at the dose of 60mg/kg considerably attenuated both

thermal and mechanical allodynia. These findings are in agreement with results of a previous study that demonstrated PTX only at higher doses (50 or 100 mg/kg) could diminish thermal and mechanical hyperalgesia in spinal nerve transection rat model (13). However, findings of this study are in contrast with another study that demonstrated systemic injection of PTX failed to alleviate mechanical allodynia (24). In the study of Liu and colleagues, PTX was given for 7 days after L5 spinal nerve transection, while in the present study rats received PTX daily for 14 days after injury in CCI model. Therefore, part of this discrepancy might be related to the differences in the duration of PTX injection and/or model nerve injury which was applied. Other findings of this study is that PTX at both low and high doses noticeably decreased thermal hyperalgesia on days of 3, 7, 11 and 14 after injury, whereas the low dose of PTX did not have any effect on thermal and mechanical allodynia.



**Figure 2.** Effects of pre-emptive and repeated administration of pentoxifylline (PTX) at doses of 30 mg/kg (PTX-30) and 60 mg/kg (PTX-60) on mechanical threshold(gram) (Von Fery test) on days of 3(A), 7(B),11(C) and 14(D) after chronic constriction injury (CCI) in rat. The data are presented as the Mean±SEM. #P<0.001 versus Sham group; \*P<0.001 versus saline-treated CCI group.

This inconsistency in response to PTX is not clear, but may be related to the difference in pathophysiology of thermal hyperalgesia and allodynia (25, 26).

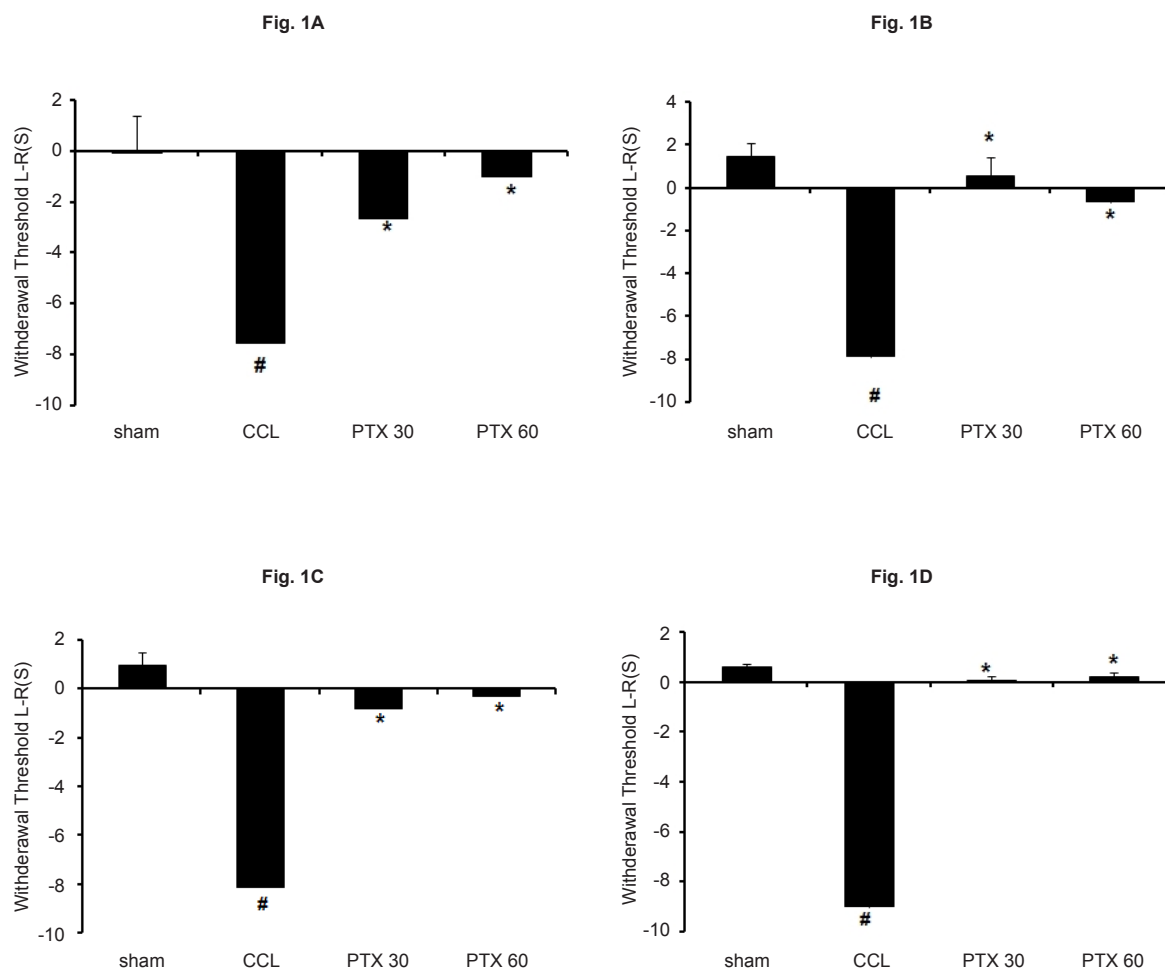
Several studies have reported that pro-inflammatory cytokines such as tumor necrosis factor (TNF- $\alpha$ ), interleukin-1 (IL-1) and nuclear factor-kappa B (NF- $\kappa$ B) may contribute to pathogenesis of neuropathic pain, with peripheral and or central nervous system mechanisms (8, 13, 27, 28). It has been shown that an inhibition of the activation of NF- $\kappa$ B reduced hyperalgesia in inflammatory and neuropathic pain models of rats (29). Another study showed that PTX dose-dependently attenuated the development of mechanical and thermal hyperalgesia via inhibiting the expression of pro-inflammatory cytokines and the activation of nuclear factor kappa B in the prefrontal brain in spinal nerve transection rat model (13). A number of studies have shown that PTX inhibited the synthesis of IL-1 and IL-6 and activation of NF- $\kappa$ B (7, 12, 13, 30). Similarly, results of our recent

study indicated that PTX (60 mg/kg) significantly reduces synthesis of TNF- $\alpha$  in ischemic brain in rats (14). Thus, it may be suggest that the attenuation of behavioral thermal, mechanical and thermal hyperalgesia by PTX after nerve injury could be due to its inhibitory effect on the production of inflammatory cytokines. Further studies are required to examine this assumption.

In conclusion, findings of this study showed that chronic administration of PTX reduces the neuropathic pain responses in a rat model of chronic constriction injury probably via inhibition of pre-inflammatory cytokines. Thus, this drug may have a potential therapeutic application in the treatment and management of neuropathic pain in humans.

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**Figure 3.** Effects of pre-emptive and repeated administration of pentoxifylline (PTX) at doses of 30 mg/kg (PTX-30) and 60 mg/kg (PTX-60) on the percent of withdrawal threshold (thermal allodynia, Acetone test) on days of 3(A), 7(B), 11(C) and 14(D) after injury in rat. The data are presented as the Mean±SEM. #P<0.001 versus Sham group; \*P<0.001 versus saline-treated chronic constriction injury (CCI).

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