Clinical Cancer Investigation Journal-ISSN: 2278-0513

Preventive Effects of Ceftriaxone and Pregabalin on peripheral Neuropathy-Induced by Cisplatinin Mice

Abstract

Peripheral neuropathy is one of the serious side effects of cisplatin that has limited its clinical use. Therefore, this study was designed to investigate the protective effects of pregabalin and ceftriaxone on cisplatin-induced peripheral neuropathy. Thirty-two swiss albino male mice were randomly divided into four equal groups including the control group, cisplatin, pregabalin, and ceftriaxone. Cisplatin (2 mg/kg) was given intraperitoneally twice a week for 6 times.

Pregabalin (30 mg/kg) and ceftriaxone (100 mg/kg) were administrated intraperitoneally once a day for the first seven days of the study, and 30 minutes before each injection of cisplatin. The tail-flick test was performed to evaluate cisplatin-induced peripheral neuropathy. The MDA and TAC levels were determined in the serum using colorimetric methods. Cisplatin meaningfully reduced the latency time in the tail-flick test as well as TAC in serum while increasing MDA. Treatment of mice with pregabalin or ceftriaxone significantly increased the pain threshold in the tail-flick test and inhibited cisplatin-induced changes in MDA and TAC. These results show that pregabalin and ceftriaxone effectively ameliorate CDDP-induced peripheral neuropathy.

Keywords:*Ceftriaxone; Cisplatin; PeripheralNeuropathy; Pregabalin*

Reza DehghanHesari[1], SamadShamsVahdati[2], MohammadbagherFazljoo[3], BahloolHabibiasl[4], RaziehTabaqi[5]

[1]Department of Traditional Medicine, School of Traditional Medicine, Tabriz University of Medical Sciences(Correspondence) Email:drdehghanh@gmail.com

[2]Department of Emergency Medicine, School of Medicine, Tabriz University of Medical Sciences

[3]Department of Traditional Medicine, School of Traditional Medicine, Tabriz University of Medical Sciences

[4]Department of Pharmacology, School of Pharmacy, Tabriz University of Medical Sciences

[5]School of Management and Medical Information sciences, Tabriz University of Medical Sciences

Introduction

Cisplatin (Cis-diammine- dichloroplatinum (II), CDDP) is a platinum-based chemotherapy agent that is now used in the treatment of diverse solid tumors such as head and neck, lung, bladder, testicular, and colorectal cancers[1, 2]. Although CDDPhas greatly increased the overall survival of cancer patients, its use has been associated with several side effects, one of the most important of which is peripheral neuropathy (PN)[3]. PN occurs in 80-90% of CDDP-treated patients and could be considered the main reason for dosage reduction or even stopping the treatment schedule[4]. Unlike the central nervous system (CNS), peripheral nerve fibers lack a blood-brain barrier (BBB) and are easily exposed to the toxic effects of CDDP[1]. Cisplatin causes peripheral neurotoxicity by mitochondrial dysfunction, oxidative stress, DNA damage, lipid peroxidation, cell membrane damage, and ultimately apoptotic cell death[5, 6].

Some previous studies have shown that antioxidant agents such as vitamin E[7], N-acetylcysteine[8], curcumin[9], caffeic acid phenethylester[10], and rutin[1] reduce or prevent cisplatin-induced neurotoxicity.

Ceftriaxone (CTX) is a beta-lactam antibiotic that specifically and irreversibly inhibits the synthesis of the bacterial cell wall by inhibiting the enzyme transpeptidase[11]. Some previous experimental studies have shown that CTX, in addition to its antimicrobial properties, has other therapeutic potentials such as anti-inflammatory, antiallodynic[12], antioxidant[13], and analgesiceffects[14]. CTX has also been shown to have neuroprotective effects against kinate by increasing the expression of the glutamate transporter GLT-1[15]. Moreover, it has been shown that CTX has potent nephroprotective activity against cisplatin, probably through its antioxidant activities[13].

Pregabalin (PGN), an anti-epileptic drug, is used for patients with neuropathic pain from fibromyalgia, diabetes, herpes zoster, or spinal cord injury.Formerexperimental studies showed that decreased oxidative neurotoxicity induced byoxaliplatin[14],CDDP[16],vincristine[17], and streptozotocin[18].In addition, the antioxidant, anti-inflammatory, and analgesic effects of PGN have been shown in various studies[19, 20].

Although several *in vivo* and *in vitro* studies have investigated the pharmacological effects of ceftriaxone, its protective effect on cisplatin-induced neurotoxicity has not been studied. Therefore, in this study, we investigated the neuroprotective effect of ceftriaxone against cisplatin-induced neurotoxicity and compared its effects with pregabalin, which is currently used to treat neuropathic pain.

Methods and Materials

Drugs and chemicals

Cisplatin, Ceftriaxone, Pregabalin,1,1,3,3 -tetraethoxypropan (TEP), Thiobarbituric acid (TBA), and Trichloroacetic acid (TCA)were obtained from Sigma-AldrichChemical Co. (Sigma, Germany). 6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), Metmyoglobin, Hydrogen peroxide, Phosphoric acid, and 2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt(ABTS)were purchased from Merck (Darmstadt, Germany).

Experimental animals

Thirty-two Swiss albino male mice (25 to 35 g) were purchased from the Pasteur Institute of Iran, Tehran, Iran, and fed using standard mouse food and tap water. The animals were kept in standard propylene cages (6 mice per cage) in a well-ventilated room with a normal temperature of 22 ± 3 °C and a 12h light / 12h dark cycle. All interventions performed on animals were according to international guidelines for the care and use of experimental animals and have been approved by the local Research Ethical Committee at Tabriz University of Medical Sciences, Tabriz, Iran (Approval No.: IR.TBZMED.VCR.REC.1398.159)

Experimental procedures

In this study, to induce peripheral neuropathy, CDDP (2 mg/kg, i.p) was injected on the seventh day of the study and then twice a week at regular intervals until the end of the fourth week. CDDP doses were adjusted weekly for each animal according to their weight change. Normal saline, CTX, or PGN were administrated Intraperitoneally (i.p) once a day for the first seven days of the study, and 30 minutes before each injection of CDDP. The animals were randomly allocated into four equal groups (n=8) as follows:

Group I (Control group): animals received NS (10 ml/kg,i.p)

Group II (Cisplatin group): animals received NS (10 ml/kg,i.p) and CDDP (six times)

Group III (Ceftriaxonegroup): animals received CTX (100 ml/kg, i.p) and CDDP (six times)

Group IV (Pregabalin group): animals received PGN (30 ml/kg, i.p) and CDDP (six times)

Tail flick test

To examine the preventive effects of ceftriaxone and pregabalin on cisplatin-induced neurotoxicity, the tail-flick test was performed before CDDP injection on days 0, 7, 15, 22, and 29th as previously described by D'Amour and Smith (1941). Briefly, the animal was inhibited inside the device and infrared thermal stimuli with an intensity of 20 W/cm2 were applied to the middle one-third of the animal's tail, and the time of tail-flick was recorded. The cut-off time of the

latency to tail flick was set up to 30 seconds to avoid any further tissue injury[21].

Sample collection

At the end of the experiment, the animals were anesthetized using xylazine/ketamine (i.p) and blood samples were taken directly from their hearts. For serum isolation, blood samples were centrifuged at 3000 rpm for 20 minutes at + 4 ° C, and the isolated sera were kept at -80 ° C until measurement of TAC and MDA.

Malondialdehyde (MDA) measurement in serum

MDA levels in the animal's serum were measured by spectrophotometry using the thiobarbituric acid reactive substance (TBARS) method [22]. Briefly, 0.5 ml of serum or TEP standard was combined with 3 ml of 1% phosphoric acid, and after stirring, 1 ml of 0.67% TBA solution was added, then the mixture was heated at 100 °C for 45 minutes and cooled using the cold-water bath. Next, the mixture was centrifuged at 3000 rpm for 10 min, and the pink color was extracted using 3 mL of normal butanol. Finally, the absorbance of the supernatant was measured at 532 nm, and the results were expressed as μmol/mL in serum.

Total Antioxidant Capacity

Serum total antioxidant capacity (TAC) was measured by a colorimetric method. In this technique, ABTS radical is incubated with methemoglobin (HX-Fe+) and hydrogen peroxide (H2O2) to produce ABTS+ radical. This cation radical is relatively stable and is green-blue, which can be measured in the range of 660 nm. The antioxidants in the sample in proportion to their concentration can inhibit the formation of this green-blue color. TAC was expressed as mmolTrolox (a vitamin E analog, as a standard) equivalent/L using the standard calibration curve [23].

Statistical analysis

The differences between obtained values (mean ± S.E.M) were tested by one-way analyses of variance (ANOVA) followed by the Tukeyposthoc test using graph pad 8.0 prism software (GraphPad Software, Inc., La Jolla, CA, USA). P values of 0.05 or less were considered to show significant differences for all comparisons made.

Results

Cisplatin induces peripheral neuropathy and it prevented by pregabalin and ceftriaxone

The results of the present study showed that intraperitoneal injection of CDDPat a dose of 2 mg/kg twice a week significantly decreased the latency time in the tail-flick test in the cisplatin group compared to the control group at the end of the second, third, and fourth weeks($P < 0.001$), which indicates that peripheral neuropathy has been successfully induced in these animals. As shown in Fig.1, pretreatment of mice with PGN(30 mg/kg, i.p), or CTX(100 mg/kg, i.p) significantly inhibited the decrease in latency time at the days of 15, 22, and 29thin cisplatin-treated mice($P < 0.05$).In addition, the results of a one-way analysis of variance showed that PGN compared to CTX significantly increased the latency time in the tail-flick test on days 22 and 29th ($P < 0.001$).

Pregabalin and ceftriaxone reduce cisplatin-induced lipid peroxidation

As shown in Fig. 2, i.p injection of cisplatin (2 mg/kg × 6 times) significantly increased serum MDA levels, an indicator of lipid peroxidation, in the CDDP group compared to the control group($P < 0.001$). The results showed the i.p administration of PGN or CTX noticeably inhibited the increase in serum MDA levels induced by cisplatin injection ($P < 0.001$). There was no significant difference in serum MDA levels between PGN and CTX groups($P > 0.05$).

Effect of cisplatin alone and in combination with pregabalin or Ceftriaxone on TAC

As illustrated in Fig. 3, intraperitoneal administration of CDDP (2 mg/kg)six times induced a significant reduction in TAC in the CDDPgroup compared to the control group ($P < 0.001$). Pretreatment of mice with PGN significantly increased the TAC in the PGN group compared to the cisplatin group ($P < 0.001$), but ceftriaxone had no significant effect on serum total antioxidant capacity in CDDP-treated mice($P > 0.05$). In addition, the total antioxidant capacity of serum in the PGN group was significantly higher than in the CTX group($P < 0.05$).

Discussion

Cisplatin, an old drug approved by the FDA, may be used alone or in combination with radiotherapy to treat solid tumors, especially refractory tumors. Although it has been largely successful in treating various cancers, its clinical use is mostly limited due to its toxicity to different organs such as renal toxicity, hepatotoxicity, and neurotoxicity[24]. Therefore, the search for novel agents that reduce CDDP organic toxicity without reducing its anticancer efficacy seems necessary. In this direction, this study was carried out to examine the preventive effects of PGN and/or CTX against CDDP-induced neurotoxicity.

Intraperitoneal treatment of mice with CDDP six times produced an oxidative stress-mediated neuropathy as marked by a significant reduction in pain tolerance threshold, augmented serum levels of MDA, and a decrease in serum

TAC. These results confirm the findings of previous studies on CDDP neurotoxicity in mice[1, 25, 26].

The study results showed that CDDP caused significant hyperalgesia in the cisplatin group at the end of the second, third, and fourth weeks and i.p administration of CTX or PGN increased the pain threshold in CDDP-treated mice. Similarly, Saadati et al. showed that i.p injection of mesna alleviates CDDP-induced thermal sensitivity[2]. Seto et al. also demonstrated that PGN significantly ameliorates CDDP-induced hyperalgesia and allodynia in rats [27]. In another study, it has been shown that different doses of ceftriaxone significantly inhibited vincristine-induced neuropathy in mice [28].

Several mechanisms have been suggested for CDDP-induced neurotoxicity; among them, oxidative stress is the most important. CDDP noticeably increases ROSs production, which leads to causes DNA and protein denaturation, cell membrane lipid peroxidation, and apoptotic neuron death[26]. The study findings showed that CDDP injection significantly increased and decreased the serum levels of MDA and TAC, respectively, and treatment of mice with PGNorCTX significantly inhibited cisplatin-induced changes in MDA and TAC.

These results confirm the finding of previous studies, which investigated the neuroprotective and nephroprotective effects of CTX against chemotherapeutic agents[11, 15, 28], this may be due to its ability to inhibit the ROS generation and increase the activity of antioxidant enzymes[13].In addition, it has been shown that cephalosporins contain thioether groups that prevent lipid oxidation by free radicals[29].

Pregabalin is an anti-epileptic drug used in the treatment of neuropathic. Several experimental studies showed that PGN has potent protective effects against oxidative neurotoxicity induced byoxaliplatin[14], CDDP[16], vincristine[17], and streptozotocin[18], these studies confirm the findings of the present study. In addition, earlier studies reported the anti-inflammatory, analgesic, and antioxidant effects of PGN[19, 20].

Conclusion

In conclusion, our results showed that pre-treatment of mice with PGN and CTX, can prevent CDDP-induced neurotoxicity and decline neuropathic pain, which may be due to their antioxidant and free radical scavenging properties.

Acknowledgments

The authors thank the Vice Chancellor for Research of Tabriz University of Medical Sciences for funding this work (research group project no.:59301) and this article was extracted from the Ph.D. thesis of Reza Dehghan.

References

1. Almutairi, M.M., et al., *Neuro-protective effect of rutin against Cisplatin-induced neurotoxic rat model.* BMC complementary and alternative medicine, 2017. **17**(1): p. 1-9.
2. Saadati, H., et al., *The Neuroprotective Effect of Mesna on Cisplatin-Induced Neurotoxicity: Behavioral, Electrophysiological, and Molecular Studies.* Neurotoxicity Research, 2021. **39**(3): p. 826-840.
3. Namvaran-Abbas-Abad, A., et al., *Cerebrolysin Ameliorates Cisplatin-Induced Neuropathic Pain in Mice: A Behavioral Study.* Journal of Experimental and Clinical Neurosciences, 2019. **6**(1).
4. Aldossary, S.A., *Review on the pharmacology of cisplatin: clinical use, toxicity, and mechanism of resistance of cisplatin.* Biomedical and Pharmacology Journal, 2019. **12**(1): p. 7-15.
5. Kanat, O., H. Ertas, and B. Caner, *Platinum-induced neurotoxicity: a review of possible mechanisms.* World journal of clinical oncology, 2017. **8**(4): p. 329.
6. Santos, N., et al., *Cisplatin-induced nephrotoxicity is associated with oxidative stress, redox state unbalance, impairment of energetic metabolism, and apoptosis in rat kidney mitochondria.* Archives of toxicology, 2007. **81**(7): p. 495-504.
7. Argyriou, A.A., et al., *A randomized controlled trial evaluating the efficacy and safety of vitamin E supplementation for protection against cisplatin-induced peripheral neuropathy: final results.* Supportive Care in Cancer, 2006. **14**(11): p. 1134-1140.
8. Zaki, S.M., et al., *N-acetylcysteine versus progesterone on the cisplatin-induced peripheral neurotoxicity.* Folia morphologica, 2018. **77**(2): p. 234-245.
9. Al Moundhri, M.S., et al., *The effect of curcumin on oxaliplatin and cisplatin neurotoxicity in rats: some behavioral, biochemical, and histopathological studies.* Journal of Medical Toxicology, 2013. **9**(1): p. 25-33.
10. Özyurt, B., et al., *The effect of antioxidant caffeic acid phenethyl ester (CAPE) on some enzyme activities in cisplatin-induced neurotoxicity in rats.* 2006.
11. Abdel-Daim, M.M., et al., *Nephroprotective efficacy of ceftriaxone against cisplatin-induced subchronic renal fibrosis in rats.* Naunyn-Schmiedeberg's archives of pharmacology, 2017. **390**(3): p. 301-309.
12. Ochoa-Aguilar, A., et al., *Ceftriaxone and clavulanic acid induce antiallodynic and anti-inflammatory effects in rats using the carrageenan model.* Journal of pain research, 2018. **11**: p. 977.
13. Abdel-Daim, M.M., et al., *The ameliorative effects of ceftriaxone and vitamin E against cisplatin-induced nephrotoxicity.* Environmental science and pollution research, 2019. **26**(15): p. 15248-15254.
14. Macaluso, A., et al., the *Analgesic effect of a single preoperative dose of the antibiotic ceftriaxone in humans.* The journal of Pain, 2013. **14**(6): p. 604-612.
15. Bajrektarevic, D. and A. Nistri, *Ceftriaxone-mediated upregulation of the glutamate transporter GLT-1 contrast neurotoxicity evoked by kainate in rat organotypic spinal cord cultures.* Neurotoxicology, 2017. **60**: p. 34-41.
16. ERTİLAV, K., *Pregabalin protected cisplatin-induced oxidative neurotoxicity in the neuronal cell line.* Journal of Cellular Neuroscience and Oxidative Stress, 2019. **11**(1): p. 815-824.
17. Nakashima, T., et al., *A case of neurotoxicity reduced with pregabalin in R-CHOP chemotherapy for diffuse large B-cell lymphoma.* Gan to Kagaku Ryoho. Cancer & chemotherapy, 2012. **39**(5): p. 809-812.
18. Jorige, A. and A. Annapurna, *Neuroprotective and antioxidant role of pregabalin in streptozotocin induced neurotoxicity.* International Journal of Pharmaceutical Sciences and Research, 2016. **7**(11): p. 4494.
19. Parasakthi, M.A. and C. Suthakaran, *Evaluation of analgesic and anti-inflammatory activity of a combination of tramadol-pregabalin in*

animal models of pain and inflammation. International Journal of Basic & Clinical Pharmacology, 2017. **6**(6): p. 1511.
20. Sałat, K., et al., *Evaluation of analgesic, antioxidant, cytotoxic and metabolic effects of pregabalin for the use in neuropathic pain.* Neurological research, 2013. **35**(9): p. 948-958.
21. Lutfy, K., et al., *Antinociceptive effects of NMDA and non-NMDA receptor antagonists in the tail flick test in mice.* Pain, 1997. **70**(1): p. 31-40.
22. Ohkawa, H., N. Ohishi, and K. Yagi, *Assay for lipid peroxides in animal tissues by the thiobarbituric acid reaction.* Analytical biochemistry, 1979. **95**(2): p. 351-358.
23. Erel, O., *A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation.* Clinical biochemistry, 2004. **37**(4): p. 277-285.
24. O'Dwyer, P.J., J.P. Stevenson, and S.W. Johnson, *Clinical status of cisplatin, carboplatin, and other platinum-based antitumor drugs.* Cisplatin: chemistry and biochemistry of a leading anticancer drug, 1999: p. 31-69.
25. Asadpour, Y., *Evaluation of the effect of Dexamethasone and Citicoline on Cisplatin-induced pain (neuropathy) in Mice*. 2019, Tabriz University of Medical Sciences, Faculty of Pharmacy.
26. Dos Santos, N.A.G., R.S. Ferreira, and A.C. Dos Santos, *Overview of cisplatin-induced neurotoxicity and ototoxicity, and the protective agents.* Food and chemical toxicology, 2020. **136**: p. 111079.
27. Seto, Y., et al., *Pregabalin reduces cisplatin-induced mechanical allodynia in rats.* Journal of pharmacological sciences, 2017. **134**(3): p. 175-180.
28. Salehian, A., *Evaluation of the effect of citalopram and ceftriaxone on neuropathy caused by vincristine in mice*. 2019, Tabriz University of Medical Sciences, Faculty of Pharmacy.
29. Anacona, J.R. and I. Osorio, *Synthesis and antibacterial activity of copper (II) complexes with sulphathiazole and cephalosporin ligands.* Transition Metal Chemistry, 2008. **33**(4): p. 517-521.

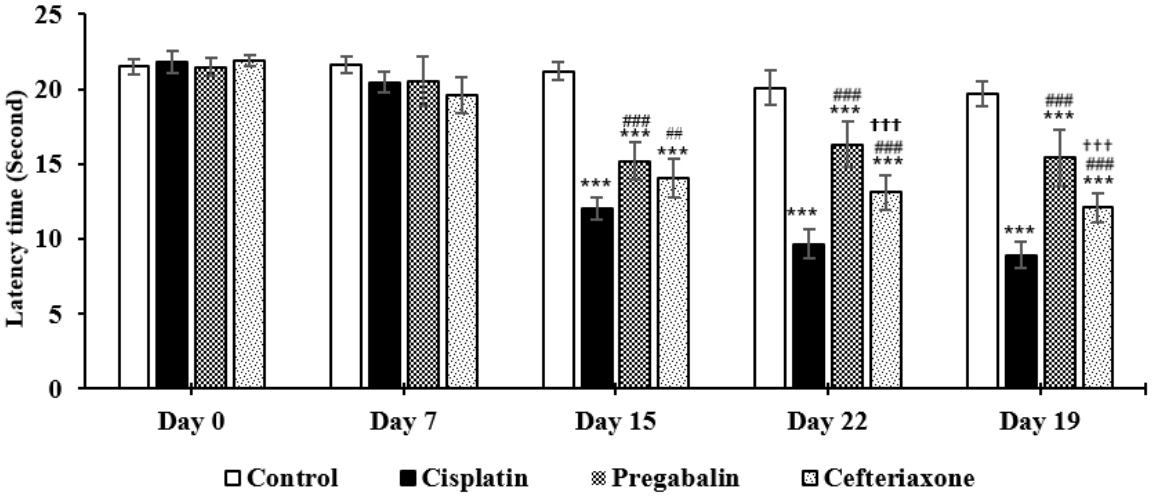

Fig. 1. Effect of Cisplatin, Pregabalin, and Ceftriaxone on tail-flick test in cisplatin-induced neuropathy in mice. Values are Mean ± SD. (n = 8). *** P < 0.001 vs. Control group, ### P < 0.001 and ## P < 0.01 vs. Cisplatin group, and ††† P < 0.001 vs. Pregabalin group.

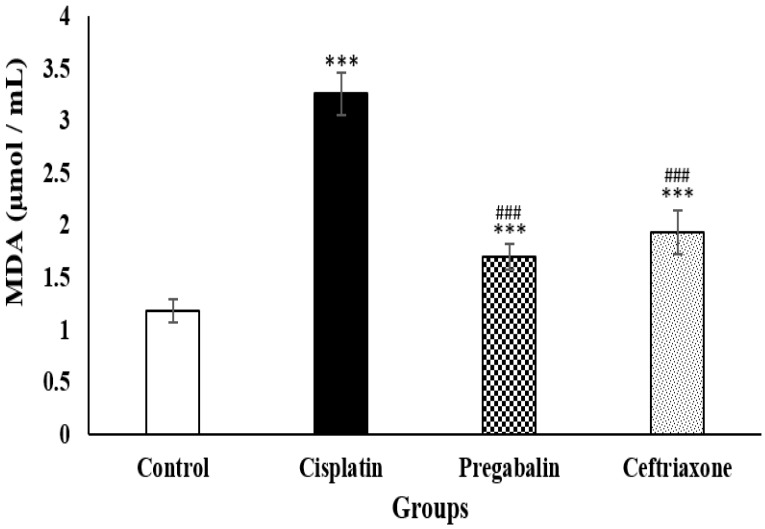

Fig. 2. Effect of pregabalin and ceftriaxone on MDA in cisplatin-induced neuropathy in mice. Values are Mean ± SD. (n = 8). *** P < 0.001 vs. Control group,and### P < 0.001 vs. Cisplatin group.

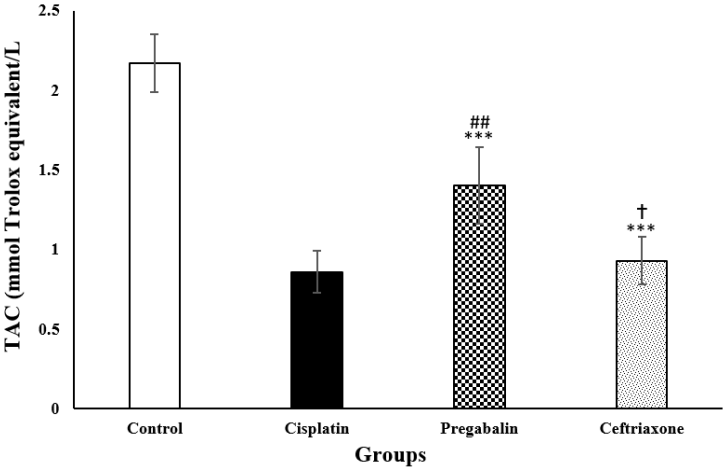

Fig. 3. Effect of pregabalin and ceftriaxone on TAC in cisplatin-induced neuropathy in mice. Values are Mean ± SD. (n = 8). *** $P < 0.001$ vs. Control group, ## $P < 0.01$ vs. Cisplatin group,and† $P < 0.05$ vs. Pregabalin group.

Table 1. Effect of Cisplatin, pregabalin, and ceftriaxone on tail-flick test in cisplatin-induced neuropathy in mice

Groups	Tail flick test's time (second)				
	Day 0	Day 7	Day 15	Day 22	Day 29
Control	21.5 ± 0.54	21.63 ± 0.57	21.2 ± 0.61	20.07 ± 1.15	19.71 ± 0.84
Cisplatin	21.8 ± 0.74	20.05 ± 0.67	11.99 ± 0.74 [a]	9.66 ± 0.95[a]	8.92 ± 0.85[a]
Pregabalin	21.47 ± 0.62	20.5 ± 1.68	15.2 ± 1.22 [a, b]	16.3 ± 1.51[a, b]	15.4 ± 1.86[a, b]
Ceftriaxone	21.89 ± 0.39	19.6 ± 1.22	14.03 ± 1.29[a, c]	13.1 ± 1.17[a, b, d]	12.09 ± 0.98[a, b, d]

Values are Mean ± SD. (n = 8). [a] $P < 0.001$ vs. Control group, [b] $P < 0.001$ and [c] $P < 0.01$ vs. Cisplatin group, and [d] $P < 0.001$vs. Pregabalin group.