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Dexamethasone and Citicoline mitigate Cisplatin-induced peripheral neuropathy: A novel experimental study in Mice

Deksametazon ve sitikolin, sisplatin kaynaklı periferik nöropatiyi hafifletir: Farelerde yeni bir deneysel çalışma

Short title: **Dexamethasone and Citicoline mitigate CisIPN**

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Abstract
Objectives: Given the rising prevalence of Cisplatin-induced peripheral neuropathy (CisIPN), investigations for alleviating its adverse effects are required. Oxidative stress and free radical development are essential pathways of CisIPN. Specifically, dexamethasone and citicoline are characterized by anti-inflammatory and antioxidant activities that might reduce CisIPN incidence and severity. The current study assessed the possible impacts of novel interventions, dexamethasone, and citicoline, on CisIPN.
Materials and Methods: Seventy-two male mice were randomly allocated into nine groups (n=8/each group). Different doses of dexamethasone (7.5, 15, 30 mg/kg, i.p.), citicoline (10, 20, 40 mg/kg, i.p.) and the combined (dexamethasone 7.5 mg/kg + citicoline 10 mg/kg, i.p.) were injected in the first three days and one day before receiving cisplatin (2 mg/kg, i.p.). The tail-flick method was used for the assessment of nociception. Besides, malondialdehyde (MDA), interleukin-1beta (IL-1β), tumor necrosis factor-α (TNF-α), total antioxidant capacity (TAC), and mice weight differences (ΔW) were measured.
Results: Different doses of dexamethasone and citicoline enhanced latency time (P<0.05). Moreover, dexamethasone 15 mg/kg diminished the level of MDA and increased TAC (P<0.05), and in 30 mg/kg, MDA was reduced (P<0.05). Besides, 20 and 40 mg/kg of citicoline reduced

MDA and elevated TAC (P<0.05), and 10 mg/kg merely reduced MDA (P<0.05). Dexamethasone in all doses declined IL-1β and TNF-α levels, and citicoline only at 40 mg/kg lessened their levels (P<0.05). Interestingly, ΔW declined more in the dexamethasone and citicoline groups than the Cisplatin group (P<0.05).
Conclusion: Dexamethasone and citicoline attenuate CisIPN through anti-inflammatory, improving antioxidant capacity, and inhibiting lipid peroxidation.
Keywords: Cisplatin, Citicoline, Dexamethasone, Oncology, Peripheral neuropathy
Öz
Amaç: Cisplatin ile indüklenen periferik nöropatinin (CisIPN) artan prevalansı göz önüne alındığında, olumsuz etkilerini hafifletmek için araştırmaların yapılması gerekmektedir. Oksidatif stres ve serbest radikal gelişimi, CisIPN'nin temel yollarıdır. Spesifik olarak, deksametazon ve sitikolin, CisIPN insidansını ve şiddetini azaltabilecek anti-inflamatuar ve antioksidan aktiviteler ile karakterize edilir. Mevcut çalışma, yeni müdahalelerin, deksametazon ve sitikolin'in CisIPN üzerindeki olası etkilerini değerlendirdi.
Gereç ve Yöntemler: Yetmiş iki erkek fare rastgele dokuz gruba ayrıldı (n=8/her grup). Farklı dozlarda deksametazon (7.5, 15, 30 mg/kg, ip), sitikolin (10, 20, 40 mg/kg, ip) ve kombine (deksametazon 7.5 mg/kg + sitikolin 10 mg/kg, ip) enjekte edildi. ilk üç gün ve sisplatin (2 mg/kg, ip) almadan bir gün önce. Nosisepsiyonun değerlendirilmesi için tail-flick yöntemi kullanıldı. Ayrıca malondialdehit (MDA), interlökin-1beta (IL-1β), tümör nekroz faktör-α (TNF-α), toplam antioksidan kapasite (TAC) ve farelerin ağırlık farklılıkları (ΔW) ölçüldü.
Bulgular: Farklı dozlarda deksametazon ve sitikolin gecikme süresini arttırdı (P<0.05). Ayrıca, 15 mg/kg deksametazon, MDA seviyesini azalttı ve TAC'yi arttırdı (P<0.05) ve 30 mg/kg'da MDA azaldı (P<0.05). Ayrıca, 20 ve 40 mg/kg sitikolin MDA'yı azalttı ve TAC'yi yükseltti (P<0.05) ve 10 mg/kg sadece MDA'yı azalttı (P<0.05). Deksametazon tüm dozlarda IL-1β ve TNF-α düzeylerini düşürdü ve sadece 40 mg/kg sitikolin düzeylerini düşürdü (P<0.05). İlginç bir şekilde, ΔW, deksametazon ve sitikolin gruplarında Cisplatin grubuna göre daha fazla azaldı (P<0.05).
Sonuç: Deksametazon ve sitikolin, anti-inflamatuar yoluyla CisIPN'yi zayıflatır, antioksidan kapasiteyi geliştirir ve lipid peroksidasyonunu inhibe eder.
Anahtar Kelimeler: Sisplatin, Sitikolin, Deksametazon, Onkoloji, Periferik nöropati

1. Introduction

Peripheral neuropathy is driven by an injury to the peripheral nervous system and can be originated from various factors, such as diabetes mellitus, vitamin insufficiency, autoimmune diseases, and particular medications. [1, 2]

Unquestionably, Chemotherapy-induced peripheral neuropathy (CIPN) is the most frequent medication-induced peripheral neuropathy that has occurred predominately with platinum-based drugs, and it affects approximately half of the patients who received these medications. [3, 4] Although the incidence of its symptoms is determined by numerous factors such as the drug's physicochemical properties, dose, duration, liver function, and age, the intolerable symptoms usually persist for several weeks after the drug discontinuation. [5]

Cisplatin (*cis*-diamminedichloroplatinum II) has been administrated to treat several solid malignancies such as ovarian, lung, and testicular carcinomas. [6] However, serious side effects, including neurotoxicity, ototoxicity, and nephrotoxicity, affect patients' quality of life, may lead to treatment discontinuation, and consequently, its clinical usage has been limited. [7, 8]

Specifically, cisplatin-induced peripheral neuropathy (CisIPN) symptoms are associated with

sensory disturbances that occur more frequently than autonomic and movement signs; its hallmarks are incorporated with weakness in the hands, gait disturbance, weakness in movements, orthostatic hypotension, and altered sexual activity. [9, 10] Interestingly, the exact mechanism of CisIPN has not been determined; however, some studies have indicated that pro-inflammatory cytokines, especially TNF-α and IL-1β, lipid peroxidation, and oxidative stress may demonstrate the accelerated factors in the incidence of CisIPN. [11-13]

Given the potential anti-inflammatory, antioxidant, and neuroprotective activities of dexamethasone and citicoline [14-19] and the lack of a study in this area, we hypothesized that they might act as potential agents to diminish CisIPN occurrence and/or severity. Insights into these aspects are expected to understand dexamethasone and citicoline impacts on CisIPN better. The present research aimed to show the possible prophylactic effects of dexamethasone and citicoline on CisIPN.

2. Material and Methods

2.1 Drugs and chemicals

Cisplatin was provided by Pfizer Inc. (NY, USA). Dexamethasone and citicoline were supplied by Iran Darou Pharmaceutical Co. (Tehran, Iran). Thiobarbituric acid, hydrogen peroxide, n-Butanol, and phosphoric acid. were obtained by Sigma-Aldrich (Sigma Aldrich Inc., Missouri, USA). Ketamine and xylazine were also purchased by Alfasan Diergeneesmiddelen B.V. (Utrecht, Netherlands)

2.2 Animals

Healthy male mice (25-35 g) were purchased from the Tabriz University of Medical Sciences animal center, housed in a standard polypropylene cage at 25 ± 2 °C temperature, and provided 12-hour light/12-hour dark intervals with *ad libitum* feeding. The Tabriz University of Medical Sciences Ethics Committee authorized the study protocols and methods (Ethical Code: IR.TBZMED.VCR.REC.1398.087, May 13, 2019), conformed to the NIH Guide for the Care and Use of Laboratory Animals (8th Edition, NRC 2011).

2.3 Experimental design

Seventy-two mice were randomly divided into the following nine groups (n=8/each group) (**Figure 1**):

(1) Control: The animals received sterile saline (10 ml/kg, i.p.) for three consecutive days and the fourth, seventh, eleventh, fourteenth, eighteenth, twenty-first, twenty-fifth days.

(2) Cisplatin: The animals in the first three days and the sixth, tenth, thirteenth, seventeenth, twentieth, and twenty-fourth days received sterile saline (10 ml/kg, i.p.). Additionally, cisplatin was injected (2mg/kg, i.p.) on the fourth, seventh, eleventh, fourteenth, eighteenth, twenty-first, and twenty-fifth days.

(3) Dex7.5: The animals in the first three days and the sixth, tenth, thirteenth, seventeenth, twentieth, and twenty-fourth days received dexamethasone (7.5 mg/kg, i.p.). Besides, on the fourth, seventh, eleventh, fourteenth, eighteenth, twenty-first, and twenty-fifth days cisplatin 2 mg/kg, i.p. was administrated.

(4) Dex15: The interventions were identical to the Dex7.5 group; however, the dexamethasone dosages were 15 mg/kg.

(5) Dex30: Similar to the conditions in the Dex7.5 group except that the dexamethasone dosages were 30 mg/kg.

(6) Cit10: Same procedure as the Dex7.5 group, but instead of dexamethasone, citicoline 10 mg/kg was administrated.

(7) Cit20: Identical procedure as the Dex7.5 group, but instead of dexamethasone, 20 mg/kg of citicoline was injected.
(8) Cit40: The condition was similar to the Dex7.5 group, but 40 mg/kg of citicoline was injected instead of dexamethasone.
(9) Dex+Cit: The conditions were the same as the Dex7.5 group; however, instead of dexamethasone, combinations of dexamethasone 7.5 mg/kg and citicoline 10 mg/kg were administrated.

In all study groups, the latency time to pain was measured using the tail-flick test on day 0 (before interventions) and repeated on days fourth, eleventh, eighteenth, twenty-fifth, and twenty-eighths.

2.4 Nociception assessment

In the tail-flick method, the thermal light (235 mW/cm^2 and 50 °C temperature) created by a lamp beam (20% intensity) from a constant distance to the 3–4 cm of animal's tail end in a special chamber (Ugo Basile 37360 Stoelting Co., IL, USA). The latency time (in seconds) was identified as the elapsed time between the beginning of the tail exposure to the thermal source and its withdrawal. [20] In the present study, the maximum time when the heat stimulus was applied to the animal's tail (Cut off time) was determined for 30 seconds to avoid tail tissue injury. Besides, stimulation was applied to successive sites from the end to the tail's beginning to increase accuracy.

2.5 Assessment Total Antioxidant Capacity (TAC)

The TAC evaluation was based on ABTS radical reduction and was performed according to the technique by Miller et al. [21] using a commercial kit (Rel Assay Diagnostics, Turkey). The results were expressed in millimoles per liter (mmol/l).

2.6 Measurement of Malondialdehyde (MDA)

MDA calculation was started by dissolving 500 µl of serum into 3 ml of 1 percent phosphoric acid. It was applied to the test tube after vortexing 1 ml of 0.67 % thiobarbituric acid solution, and after full vortexing, it was put for 45 minutes in a laboratory water bath. The test tubes were cooled under cold water after the necessary time; 3 ml of normal butanol was applied and vortexed for 2 minutes, centrifuged for ten minutes at 3000 rpm; eventually, the supernatant was collected for calculating light absorbance at 532 nm. [22] The results were reported in nanomoles per liter (nmol/l).

2.7 Weight changes

The animal weights were measured at baseline (day 0) and repeated on days 7, 14, and 28 by an analytical balance (Libror AEU-210, Shimadzu, Japan); their weight changes for each study group were recorded and compared.

2.8 Assay of proinflammatory cytokines

Mice were anesthetized intraperitoneally (i.p.) by administering a combination of ketamine (50 mg/kg) and xylazine (5 mg/kg); blood samples were obtained from their abdominal aorta and centrifuged at 10000 rpm for ten minutes at 4 °C and collected supernatants were frozen at −80 °C to measure the levels of proinflammatory cytokines. Consequently, tumor necrosis factor-α (TNF-α) and interleukin-1beta (IL-1β) levels were assessed using Enzyme-linked Immunosorbent Assay (ELISA) kits (Bender Medsystems, Vienna, Austria). Briefly, In a 96-well plate, 50-ml of standards were inserted, polyclonal antibodies were separately added to all wells, and their surfaces were coated and incubated at room temperature (25 °C) for 2 hours. Followed by washing, the wells were filled with streptavidin-HRP and incubated at room

temperature. Afterward, a colored product parallel to the amount of IL-1β and TNF-α in the sample was formed by adding tetramethylbenzidine substrate solution to all wells. Finally, to prevent the enzyme reaction, the stop solution was applied to each well, and the relative absorbance of TNF-α and IL-1β were measured spectrophotometrically at 450 nm (Synergy HT, BioTek, USA). The results were standardized to the amount of protein in each sample and expressed as a picogram per milligram (pg/mg) of protein. [23]

2.9 Statistical analysis

Statistical analyses were conducted using SPSS software 25 (SPSS Inc., Chicago, Illinois). Data from experiments are presented as Mean ± standard errors of means (SEM). An ANOVA test with the *Tukey* post hoc analysis was utilized to assess various treatment regimens' efficacy between study groups. A $P<0.05$ value was assumed to demonstrate a statistically significant difference.

3. Results

3.1 Different interventions and CisIPN

The comparative effects of different interventions on CisIPN pain hypersensitivity are presented in **Figure 2**. Cisplatin injection in the Cisplatin group on days 25 and 28 significantly diminished the latency time compared with the Control group ($P<0.01$) (**Figure 2A**). Administration of dexamethasone with 7.5 and 15 and mg/kg (Dex7.5 and Dex15 groups) on days 11, 18, 25, and 28 ($P<0.001$) and 30 mg/kg (Dex30 group) on the fourth day ($P<0.05$) and days 25 and 28 ($P<0.001$) could significantly increase the latency time to pain compared with the Cisplatin group (**Figure 2B).** Besides, citicoline 10 mg/kg on the fourth day ($P<0.01$) and on days 11, 18, 25, and 28 ($P<0.001$), also at doses 20 and 40 mg/kg on days 28, 25, 18, 11 ($P<0.001$) substantially increased pain latency time in comparison to the Cisplatin group (**Figure 2C)**. Moreover, co-administration of dexamethasone and citicoline (Dex+Cit group) on days 11 ($P<0.01$), 18 ($P<0.05$), 25, and 28 ($P<0.001$) dramatically improved latency time in comparison with the Cisplatin group; however, on day 28 ($P<0.01$) compared with dexamethasone group (Dex7.5) and on days 11 and 25 ($P<0.01$), 18 and 28 ($P<0.001$) in comparison with citicoline group (Cit10) the latency time was declined (**Figure 2D**).

3.2 Changes in mice weights

Administration of cisplatin in the Cisplatin group reduced body weight differences (ΔW) compared to the Control group (-1.07±0.09 vs. 0.14±0.11; $P>0.05$). Also, dexamethasone at doses 7.5 and 30 (Dex7.5 and Dex30 groups) decreased ΔW significantly compared to the Cisplatin group (-3.03±0.2 vs. -1.07±0.09; $P<0.001$, and -6.29±0.45 vs. -1.07±0.09; $P<0.001$, respectively). On the other hand, citicoline at all doses declined ΔW meaningfully in comparison to the Cisplatin group (Cit10, -5.57±0.01 vs. -1.07±0.09; $P<0.001$, Cit20, -4.34±0.07 vs. -1.07±0.09; $P<0.001$, and Cit40, -3.11±0.55 vs. -1.07±0.09; $P<0.01$). Moreover, the combination of dexamethasone and citicoline (Dex+Cit group) reduced ΔW notably compared with the Cisplatin group (-4.5±0.25 vs. -1.07±0.09; $P<0.001$), Cit10 group (-4.5±0.25 vs. -5.57±0.01; $P<0.001$), and Dex7.5 group (-4.5±0.25 vs. -3.03±0.2; $P<0.01$) **(Table 1)**.

3.3 TAC and MDA levels

Cisplatin administration in the Cisplatin group increased MDA significantly compared to the Control group (2.52±0.28 vs. 1.64±0.09; $P<001$). The injection of dexamethasone (7.5, 15, and 30 mg/kg) elevated TAC and declined MDA levels in comparison to the Cisplatin group; however, merely at the 15 mg/kg (Dex15 group) showed significant differences ($P<0.05$). Also, the reduced MDA level in the Dex30 group was meaningfully compared with the Cisplatin group

(P<0.05). Similarly, citicoline at all doses elevated TAC and diminished MDA levels in comparison to the Cisplatin group; notably, these outlined changes have occurred at doses 10 mg/kg (TAC, 0.57±0.05 vs. 0.54±0.04; P>0.05, MDA, 1.46±0.08 vs. 2.52±0.28; P<0.01), 20 mg/kg (TAC, 0.75±0.04 vs. 0.54±0.04; P<0.05, MDA, 1.73±0.17 vs. 2.52±0.28; P<0.05), and 40 mg/kg (TAC, 0.82±0.04 vs. 0.54±0.04; P<0.001, MDA, 1.8±0.15 vs. 2.52±0.28; P<0.05). Moreover, the co-administration of dexamethasone and citicoline (Dex+Cit group) enhanced TAC and reduced MDA levels compared with the Cisplatin group (0.69±0.05 vs. 0.54±0.04; P<0.05 and 1.55±0.21 vs. 2.52±0.28; P<0.05, respectively) **(Table 2)**.

3.4 TNF-α and IL-1β levels

Figure 3 shows that TNF-α levels in the Cisplatin group were significantly enhanced compared with the Control group (P<0.01). Administration of dexamethasone at doses 7.5 mg/kg (Dex7.5), 15 mg/kg (Dex15), and 30 mg/kg (Dex30) significantly declined the TNF-α levels compared with the Cisplatin group (P<0.05, P<0.05, and P<0.01, respectively). Besides, citicoline showed a considerable reduction at dose 40 mg/kg (Cit40) in comparison with the Cisplatin group (P<0.05). The combination of dexamethasone and citicoline (Dex+Cit) did not demonstrate a significant TNF-α levels change compared to the Cisplatine group; nonetheless, its levels diminished considerably compared with the Cit10 group (P<0.05).

As shown in **Figure 4**, the IL-1β level was enhanced meaningfully in the Cisplatin group compared to the Control group (P<0.001). Additionally, dexamethasone at all doses 7.5 mg/kg (Dex7.5), 15 mg/kg (Dex15), and 30 mg/kg (Dex30), significantly diminished the IL-1β levels in comparison with the Cisplatin group (P<0.01, P<0.01, and P<0.001, respectively). Moreover, citicoline only at dose 40 mg/kg indicated a significant reduction in IL-1β level compared with the Cisplatine group (P<0.05). The combination of dexamethasone and citicoline (Dex+Cit) did not reveal IL-1β changes considerably than the Cisplatin group; however, its levels decremented significantly as compared with the Cit10 group (P<0.01).

4. Discussion

The effectiveness of different doses of dexamethasone and citicoline on CisIPN in healthy male mice was evaluated. Administration of dexamethasone in different doses indicated a significant preventive effect on raising latency times at doses 7.5 and 15 mg/kg from the eleventh day, and in 30 mg/kg dose from the twenty-fifth day, compared with the Cisplatin group. Citicoline in all doses significantly increased the latency time from the 11th day than the Cisplatin group. Moreover, co-administration of dexamethasone and citicoline (Dex+Cit group) indicated their significant protective effects from the 11th day compared to the Cisplatin and Cit10 groups; however, in comparison with the Dex7.5 group, a considerable difference was only obtained on the 28th day. Specifically, neither of the interventions (dexamethasone and citicoline) demonstrated a dose-dependent efficacy on latency time; nonetheless, their beneficial impacts on MDA and TAC levels were dose-dependent.

Conventional pharmacological treatments for CisIPN, including tricyclic antidepressants (TCAs), some anticonvulsants (pregabalin and gabapentin), opioids, and nonsteroidal anti-inflammatory drugs (NSAIDs), present various side effects, and their efficacy remain unclear. Significantly, inflammation, oxidative stress, altered calcium channel activity, mitochondrial damage, and serotonergic system are associated mechanisms that have been identified in developing CisIPN. [24]

In a study by Takasaki et al., which examined the antagonist effects of glucocorticoid receptors on allodynia and hyperalgesia in mice-induced neuropathic pain, their results showed that dexamethasone exerted its anti-inflammatory and immunosuppressive effects via spinal

glucocorticoid receptors that play a beneficial target in treating peripheral neuropathy. [25] A meta-analysis study, which included 32 studies (2697 patients), presented that dexamethasone partnered dosing could decline bortezomib-induced peripheral neuropathy severity. [26] Moattari et al. found that dexamethasone enhanced sciatic nerve function, regeneration, and histomorphological characteristics followed by sciatic nerve dissection surgery. [27] Additionally, retrospective analyses that included 190 patients demonstrated that dexamethasone could diminish CIPN severity. [28] Findings from a systematic review of dexamethasone combination therapy with thalidomide (twelve studies, including 451 patients with multiple myeloma) indicated that dexamethasone could significantly reduce peripheral neuropathy. [29]

Numerous studies have been implied that citicoline demonstrated its neuroprotective properties through increasing Sirtuin-1 [30, 31], acetylcholine [32, 33], and serotonin [34], decreasing glutamate levels [17], anti-inflammation activity via blocking phospholipase A2, and diminish reactive oxygen species (ROS) generation. [14] Besides, in an experiment by Bagdas et al., they used the Randall–Sellito test to evaluate the pain threshold, which citicoline significantly elevated the pain threshold through central opioid receptors in a neuropathic pain rat model. [35]

Total antioxidant capacity (TAC) has been used to determine antioxidant activity and antioxidant response to generated free radicals in particular diseases. Low TAC levels also could be inferred from oxidative stress or enhanced exposure to oxidative stress-induced tissue injuries. [36] Additionally, MDA levels have been measured as the primary lipid peroxidation indicator, and its elevated levels are associated with cell damages. [37] Therefore, measuring TAC and MDA are essential factors for assessing the proposed interventions on CisIPN. Kamisli et al. [38] demonstrated that cisplatin triggered lipid peroxidations and diminished antioxidant defense mechanisms in the brain and sciatic nerve. In the current study, cisplatin enhanced MDA and reduced TAC levels may induce decrement antioxidant nerve cells' capacity. Alternatively, dexamethasone and citicoline in the relevant groups elevated TAC and diminished MDA levels compared with Control and Cisplatin groups, which exert profitable outcomes on CisIPN through antioxidant activities.

Considering the glucocorticoids' catabolic impact on skeletal muscle [39], it is not surprising that the Dex30 group (dexamethasone 30 mg/kg) demonstrated the lowest ΔW compared with other study groups. Moreover, the ΔW lessened in all cisplatin-treated groups (groups 2-9) compared with the Control group, implies the induced weight loss by cisplatin in the abovementioned groups. Precisely, cisplatin activates the central nucleus of the amygdala, lateral parabrachial nucleus, and nucleus tractus solitarius neurons'; these regions may trigger weight loss through CGRP/glutamatergic signaling. [40] However, it remains unclear in the current study that citicoline administration (Cit10, Cit20, Cit40, and Dex+Cit groups) presented more diminished ΔW than the Cisplatin group. Besides, we have not found any study that showed citicoline could attenuate body weights; interestingly, some studies have revealed that citicoline mitigated body weight loss. [41, 42]

It has been indicated that proinflammatory cytokines such as IL-1β and TNF-α have a significant role in cisplatin-induced vestibular damage. [43] Also, their expressions increased in cisplatin-induced peripheral nerve injuries, contributing to peripheral neurons excitability and sensitization by mediating tetrodotoxin-resistant sodium channels in nociceptors. [44, 45] Following immune cell activation, IL-1β and TNF-α generating inflammation, they contribute to the development of peripheral sensitization through producing nerve growth factor (NGF) and prostaglandin E_2 (PGE_2), which triggers pain hypersensitivity in nociceptor dorsal root ganglion (DRG) neurons. Therefore, inhibiting the synthesis or development of IL-1β and TNF-α reaches

anti-inflammatory impacts and acts as an analgesic for inflammatory pain. [46] Our results demonstrated that cisplatin elevated IL-1β and TNF-α levels, and administration of dexamethasone and citicoline attenuated them in the treatment groups (Dex7.5, Dex15, Dex30, Cit10, Cit20, Cit40, and Dex+Cit), suggesting that they have potential activity against CisIPN. The present study revealed that cisplatin enhanced MDA, TNF-α, and IL-1β levels; and it declined TAC and pain hypersensitivity; nevertheless, in the treatment groups, MDA and proinflammatory cytokines (IL-1β and TNF-α) diminished, and TAC and hypersensitivity to pain elevated. The neuroprotective effect of dexamethasone and citicoline appear to be conferred via anti-inflammatory (declining IL-1β and TNF-α levels) and antioxidant (enhancing the antioxidative capacity and diminishing lipid peroxidation) activities. To our knowledge, this was the first attempt to apply dexamethasone and citicoline in CisIPN, and the evaluation of these two medications is assumed to be novel because it has not been administrated in the treatment of CisIPN.

5. Conclusions

The most critical finding of this research is that dexamethasone and citicoline administration, along with cisplatin, increased the latency time and TAC, declined TNF-α and IL-1β, and attenuated lipid peroxidation by reduced MDA levels. We observed that the relevant groups' proposed interventions were better than the Control and Cisplatin groups in all the experiments. The experiment results conclude that since dexamethasone and citicoline have beneficial neuroprotective effects on CisIPN fundamental mechanisms, clinical studies should be conducted to validate these effects in patients with CisIPN symptoms.

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Tables

Table 1: Changes in mice weights between study groups

Groups	Day 0	Day 7	Day 14	Day 28	ΔW
Control	28±1.44	29.57±1.41	28.71±1.47	28.14±1.33	0.14±0.11
Cisplatin	27.28±0.68	27.28±0.56	26.85±0.63	26.21±0.77	-1.07±0.09
Dex7.5	30.12±1.5	28.37±1.33	28.12±1.29	27.09±1.3	-3.03±0.2***
Dex15	26.85±0.34	26±0.81	25.57±1.13	23.81±1.35	-3.04±1.01**
Dex30	33.14±0.4	31.85±0.4	28.07±0.78	26.85±0.85	-6.29±0.45***
Cit10	28.14±1.31	26.71±1.2	25.14±1.12	22.57±1.32	-5.57±0.01***
Cit20	27.5±0.61	25.83±0.6	24.08±0.63	23.16±0.54	-4.34±0.07***
Cit40	29.14±1.03	27.57±1.2	27.12±1.3	26.03±1.58	-3.11±0.55**
Dex+Cit	25.83±1.24	25.66±1.4	23.33±1.54$	21.33±1.49$$	-4.5±0.25*** $$ ŦŦŦ

Data are provided as Mean ± SEM, g. *n*=8/each group. (ΔW, weight changes ($W_{in\ day\ 28}$-$W_{in\ day\ 0}$)). Dex7.5, dexamethasone 7.5 mg/kg; Dex15, dexamethasone 15 mg/kg; Dex30, dexamethasone 30 mg/kg; Cit10, citicoline 10 mg/kg; Cit20, citicoline 20 mg/kg; Cit40, citicoline 40 mg/kg; Dex+Cit, dexamethasone 7.5 mg/kg and citicoline 10 mg/kg. ***P*< 0.01 and ****P*<0.001 as compared with the Cisplatin group. $ *P*< 0.05 and $$ *P*<0.01 as compared with the Dex 7.5 group. ŦŦŦ *P*<0.001 as compared with the Cit10 group.

Table 2: TAC and MDA levels in the study groups

Groups	TAC (*mmol/L*)	MDA (*nmol/L*)
Control	0.6±0.02	1.64±0.09
Cisplatin	0.54±0.04	2.52±0.28$^{\#\#}$
Dex7.5	0.58±0.03	2.02±0.3
Dex15	0.65±0.03*	1.85±0.16*
Dex30	0.66±0.37	1.65±0.06*
Cit10	0.57±0.05	1.46±0.08**
Cit20	0.75±0.04*	1.73±0.17*
Cit40	0.82±0.04***	1.8±0.15*
Dex+Cit	0.69±0.05*	1.55±0.21*

Data are presented as Mean ± SEM. *n*=8/each group. Dex7.5, dexamethasone 7.5 mg/kg; Dex15, dexamethasone 15 mg/kg; Dex30, dexamethasone 30 mg/kg; Cit10, citicoline 10 mg/kg; Cit20, citicoline 20 mg/kg; Cit40, citicoline 40 mg/kg; Dex+Cit, dexamethasone 7.5 mg/kg and citicoline 10 mg/kg. *P< 0.05, **P< 0.01, and ***P<0.001 as compared with the Cisplatin group. ##P<0.01 as compared with the Control group.

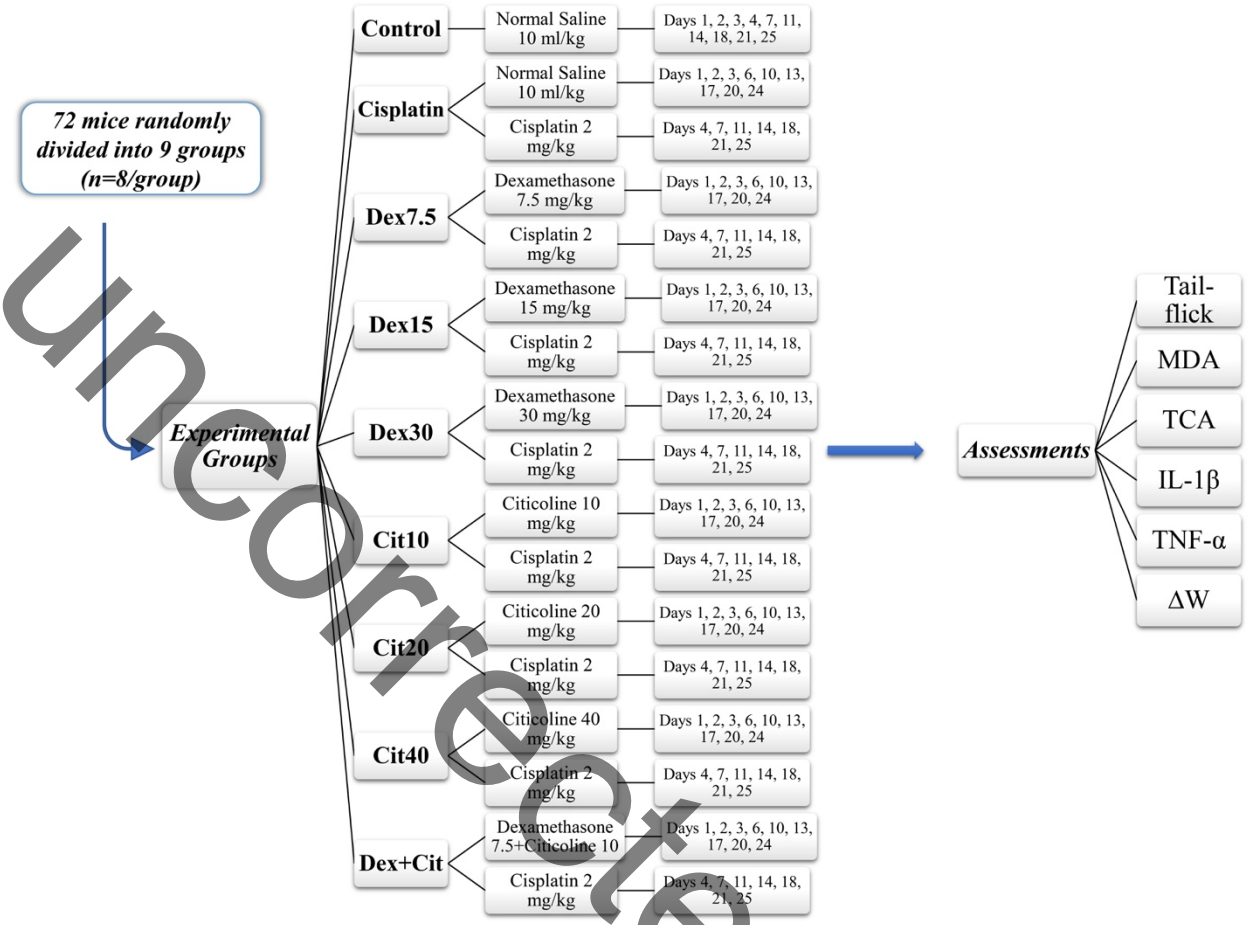

Figure 1: Functional diagram of the experimental groups and the assessments. In all figures, Dex7.5, dexamethasone 7.5 mg/kg; Dex15, dexamethasone 15 mg/kg; Dex30, dexamethasone 30 mg/kg; Cit10, citicoline 10 mg/kg; Cit20, citicoline 20 mg/kg; Cit40, citicoline 40 mg/kg; and Dex+Cit, dexamethasone 7.5 mg/kg and citicoline 10 mg/kg. Moreover, MDA, malondialdehyde; IL-1β, interleukin-1beta; TNF-α, tumor necrosis factor-α; TAC, total antioxidant capacity; and ΔW, mice weight differences.

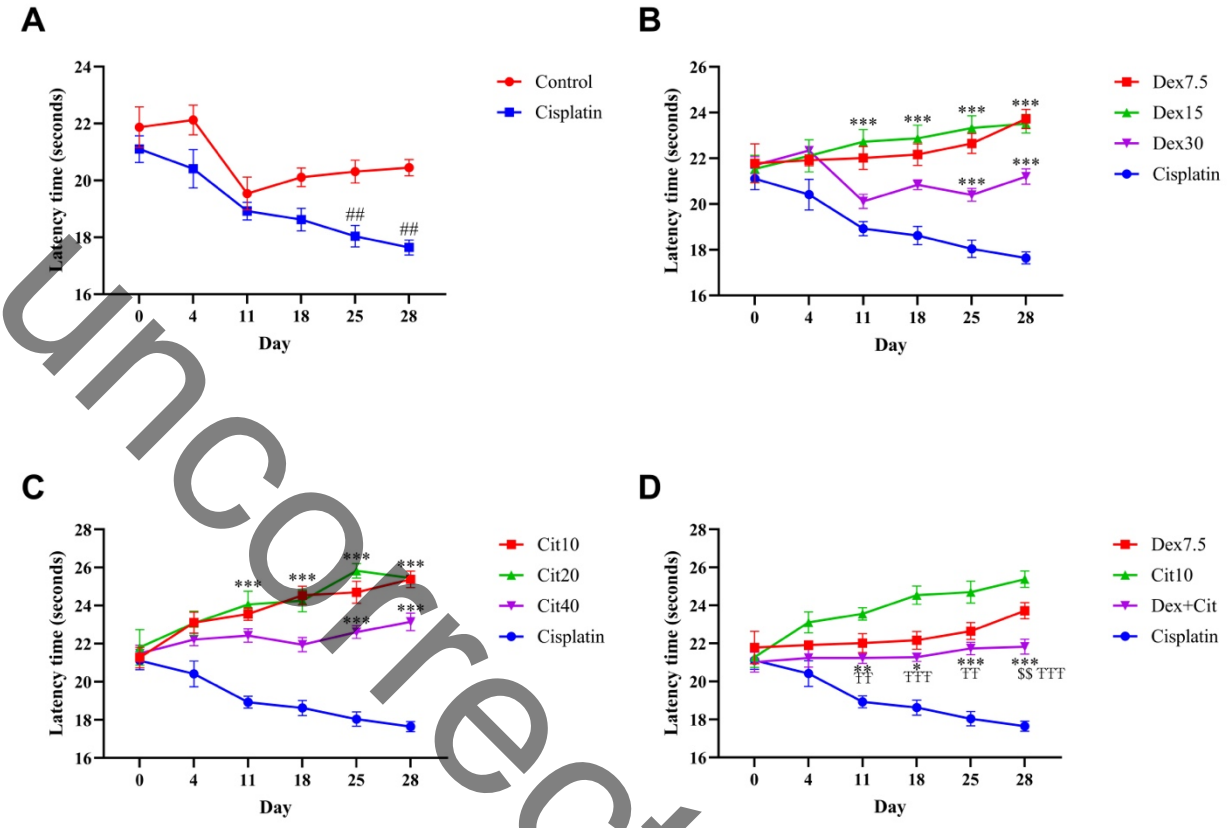

Figure 2: The effect of different interventions on latency times in study groups. (A) comparison between the Control and Cisplatin group. (B) comparing dexamethasone injected groups (Dex7.5, Dex15, Dex30, and Dex+Cit) with the cisplatin group. (C) comparing with citicoline injected groups (Cit10, Cit20, Cit40, and Dex+Cit) and the Cisplatin group. (D) comparison of the combination group (Dex+Cit) with Dex7.5 and Cit10 groups. Data are provided as Mean ± SEM (*n*=8/each group). Statistical analyses were conducted using one-way *ANOVA* with the *Tukey* test post hoc test. **P*<0.05, ***P*<0.01, and ****P*<0.001 compared with the Cisplatin group. ##*P*<0.01 as compared with the Control group. $$ *P*<0.01 as compared with the Dex7.5 group. ŦŦ *P*<0.01 and ŦŦŦ *P*<0.001 as compared with the Cit10 group.

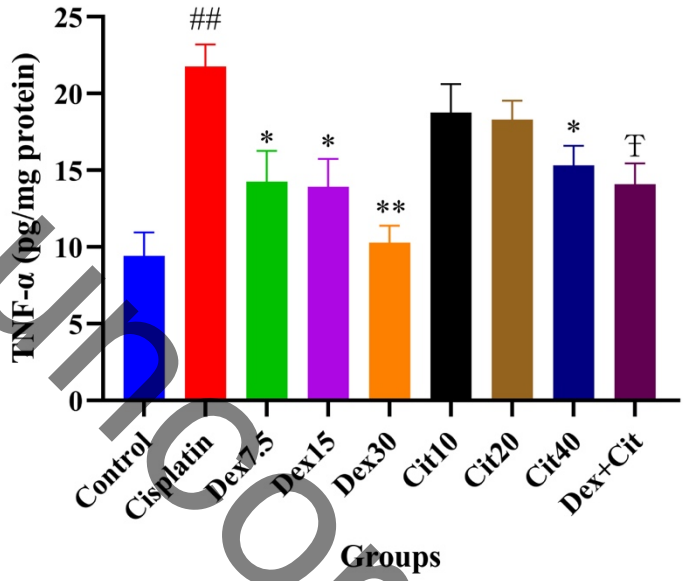

Figure 3: The levels of TNF-α in different groups. Data are presented as Mean ± SEM (*n*=8/each group). **P*<0.05 and ***P*<0.01 compared with the Cisplatin group. ##*P*<0.01 as compared with the Control group. Ŧ *P*<0.05 as compared with the Cit10 group.

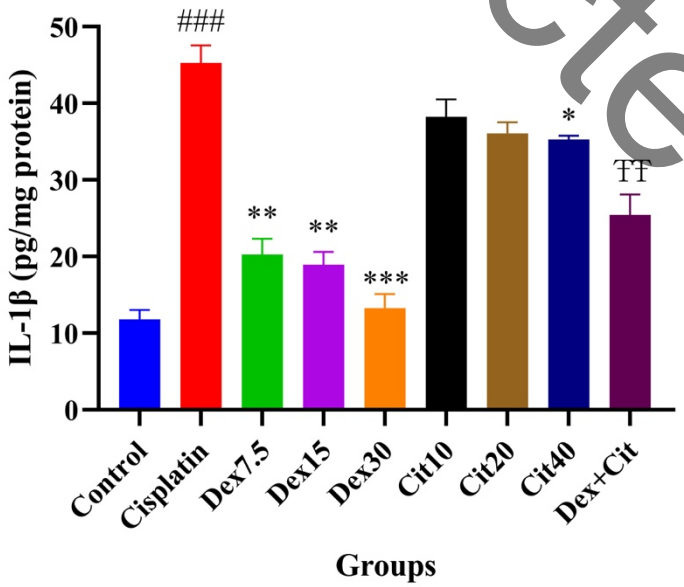

Figure 4: The IL-1β levels in different groups. Data are provided as Mean ± SEM (*n*=8/each group). **P*<0.05, ***P*<0.01, and ****P*<0.001 compared with the Cisplatin group. ###*P*<0.001 as compared with the Control group. ŦŦ *P*<0.01 as compared with the Cit10 group.