

## Original Article

### Cerebrolysin Ameliorates Cisplatin-Induced Neuropathic Pain in Mice: A Behavioral Study

Ali Namvaran-Abbas-Abad<sup>a</sup>, Fereshteh Farajdokht<sup>b</sup>, Saeed Sadigh-Eteghad<sup>b</sup>, Javad Mahmoudi<sup>b</sup>, Seyed Mahdi Vatandoust<sup>b,\*</sup>

<sup>a</sup>Hematology and Oncology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

<sup>b</sup>Neurosciences Research Center (NSRC), Tabriz University of Medical Sciences, Tabriz, Iran

#### Abstract

**Objective:** Cisplatin (Cis) is a potent chemotherapeutic agent in clinical use which is associated with nephrotoxicity and neuropathic pain possibly through inflammatory response. Cerebrolysin (CBL), a mixture of neurotrophins, has analgesic and anti-inflammatory properties. The aim of the present study was to investigate the effects of cerebrolysin on cisplatin-induced neuropathic pain in mice.

**Materials and Methods:** Mice were randomly divided into five groups: Control, Cis, Cis + CBL, Cis + Vitamin E (Vit E), and Cis + Morphine. CBL and Vit E were injected for four consecutive days following a single injection of Cis. On day 4, the mice were subjected to three behavioral tests: cold plate, hot plate, and formalin.

**Results:** The results showed that mice treated with CBL had higher withdrawal thresholds for both the hot plate and cold plate tests and displayed lower hyperalgesia-related behaviors for the formalin test compared to the Cis group. Moreover, CBL induced higher anti-nociceptive effects than Vit E and lesser effects than morphine in both hot plate and cold plate tests. Furthermore, the analgesic effect of CBL was similar to the morphine effect in the late phase of the formalin test.

**Conclusion:** These results indicate that CBL has an anti-nociceptive effect against cisplatin-induced neuropathic pain.

**Keywords:** Cisplatin, Neuropathic Pain, Cerebrolysin, Hyperalgesia

#### Correspondence

Seyed Mahdi Vatandoust  
Neurosciences research center (NSRC),  
Tabriz University of Medical Sciences,  
Tabriz, Iran. Tel/Fax: +984133340730.  
Email: [sm.vatandoust@gmail.com](mailto:sm.vatandoust@gmail.com)

Received: 2018-10-10

Accepted: 2018-11-01

DOI: [10.13183/jecns.v6i1.85](https://doi.org/10.13183/jecns.v6i1.85)

©2019 Swedish Science Pioneers, All rights reserved.

#### Introduction

Neuropathic pain is a chronic pain condition affecting millions of individuals worldwide [1]. This condition is caused by different ailments, such as diabetes [2], injuries, and interventions [3-5], that affect the somatosensory system [1]. Chronic pain results in a great many of these cases [6]. Chemotherapy-induced peripheral neuropathy (CIPN) is a troublesome chronic condition experienced by 30% to 40% of patients undergoing sequential chemotherapy, and it remains as a common dose-limiting side effect of chemotherapeutic agents [3, 7]. Moreover, CIPN may have negative consequences on treatment procedures [8], quality of life, and daily activities [3, 7, 8]. Unfortunately, little is known about CIPN's exact pathophysiology, and available treatments have a restricted efficiency in relieving pain [8].

Cisplatin is a potent chemotherapeutic agent used to treat a wide range of solid tumors, including testicular, ovarian, and

bladder cancers [9]. The administration of cisplatin is also associated with neurotoxicity via irreversible axonal loss [10] and with neuropathic pain in distal extremities [11]. Cisplatin is extensively used as an animal model of neuropathic pain to study the effects of neuroprotective substances [11-13]. However, pain management remains a major challenge that restricts cisplatin use in clinical settings. In addition, currently available therapies are not able to effectively control neuropathic pain symptoms [11, 14].

Cerebrolysin (CBL) is a purified mixture containing a fraction of free amino acids and some brain growth factors [15], including brain-derived neurotrophic factor (BDNF), glial cell line-derived neurotrophic factor (GDNF), nerve growth factor (NGF), and ciliary neurotrophic factor (CNTF) [16]. Evidence shows that CBL is capable of partially crossing the blood-brain barrier because it contains low molecular weight peptides. Previous research has shown that CBL improves different neurological conditions,

such as stroke, dementia, diabetic neuropathic pain, spinal cord injury, and migraine-associated symptoms [18-23]. Moreover, it promotes neuronal cells proliferation and exerts protective effects on different neuronal populations [16, 17, 21, 22]. GDNF, as a component of CBL, has potent analgesic effects in animal models of neuropathic pain [24].

Given this, it seems that CBL may provide benefits in treating complicated conditions that affect neurons' viability and functions. Based on this evidence, the current study examined the effects of CBL on cisplatin-induced neuropathic pain in mice.

## Material and Methods

### Animals

Male BALB/c mice weighing 25-30 g were obtained from the laboratory animal unit of the Tabriz University of Medical Sciences. Animals were kept, 5 per cage, under standard laboratory conditions with a 12 hour diurnal cycle (lights on at 7:00 am; lights off at 9:00 pm), at 22-25°C temperature and 45-55% humidity. All mice had free access to standard mouse food and water.

All experiments were carried out in accordance with the Guide and Use of Laboratory Animals (NIH; Publication No. 85-23, revised 1985) and confirmed by the Ethical Committee for Animal Experimentation of the Tabriz University of Medical Sciences.

### Experimental design

After a week of habituation, animals were randomly assigned to one of the following groups (n=18 per group): Control (0.2 ml of 0.9 % saline through intraperitoneal [i.p.] route); Cis (single intravenous [i.v.] injection of 2 mg/kg Cis through tail vein); Cis + CBL (i.p. injection of CBL 5 ml/kg, for 4 days following receiving single i.v. dose of Cis); Cis + Vit E (i.p. injection of 5 g/kg Vit E for 4 days following single i.v. dose of Cis); and Cis + Morphine (single i.p. injection of 10 mg/kg morphine following single i.v. dose of Cis). All drugs were freshly prepared on the day of use and were administered at 10 ml/kg volume. Four days after the initiation of the injection procedure, animals in all groups were divided into 3 subgroups and formalin, hot plate, and cold plate tests were administered. The last dose of CBL was administered 30 minutes before behavioral tests and Vit E was injected 60 minutes before experiments. In the Cis + Morphine group, animals received a single injection of morphine (10 mg/kg, i.p.) before being subjected to the tests. Doses of chemicals were adopted from previous studies [11, 13, 25-29].

### Chemicals

Cisplatin was purchased from the Mylan Company, France. CBL was purchased from EBEWE Pharma GmbH, Austria. Vitamin E and morphine sulfate were purchased from Darupakhsh Company, Iran.

### Behavioral assessments

All behavioral tests were performed under blinded conditions between 8:00 am and 2:00 pm in a quiet room. All efforts were

made to reduce animal suffering and minimize the number of animals used.

### Cold plate assay

Mice were brought to the testing room and allowed to habituate for handling and testing before being individually tested. Cold hyperalgesia was evaluated on a cold plate. Briefly, animals were gently placed in a cylinder on a cold plate maintained at a temperature of  $-4.2 \text{ }^{\circ}\text{C} \pm 0.2 \text{ }^{\circ}\text{C}$ . A covered transparent Plexiglas cylinder (15 cm in diameter and 20 cm height) was used to prevent escape. Latency time was recorded for the first stamp or lift of the hind paw. Movements associated with locomotion, including coordinated movement of all four limbs, were excluded. The time of the brisk response was interpreted as the latency for cold pain withdrawal. A maximum cut-off time of 150 seconds was used to prevent tissue damage; any response with a latency greater than 150 seconds was considered non-painful. Each mouse was tested once for any given test to avoid possible tissue damage and analgesic effects that could be produced by repeated exposure to different tests [12, 30].

### Hot plate assay

Nociception was evaluated using the hot plate test as described previously [31]. In brief, the system consisted of an electrically heated surface and a Plexiglas tube (20 cm high  $\times$  25 cm diameter) to restrict the animals to the heated surface. Habituation was used to minimize learning effects prior to each test. The temperature was kept at  $48.0 \pm 0.5 \text{ }^{\circ}\text{C}$ . The animal was placed on the hot plate, and the time between the placement of mice on the hot plate and the occurrence of the hind paw licking and/or jumping from the surface was recorded as response latency by a stopwatch. A maximum of a 30-second cut-off time was used to prevent tissue damage.

### Formalin Test

The formalin test was performed in a temperature-controlled (20-22  $^{\circ}\text{C}$ ) and a quiet room. Animals were placed in an observation container to adapt to the new environment for 15 minutes prior to the test. Then, 20  $\mu\text{l}$  of 5% formalin (Merck, Germany) was injected subcutaneously into the hind paw by an insulin syringe, in a restrainer. Animals were immediately returned to the observation chamber, and the formalin-evoked nociceptive response either in early (0-5 minutes post-injection) or late phases (20-60 minutes) was recorded over one hour [32].

### Data analysis

Data are presented as means  $\pm$  standard error of the mean (SEM) and were analyzed using SPSS version 21 statistical software. One-way ANOVA was used, followed by Tukey's post hoc test. The level of statistical significance was set at  $p < 0.05$ .

## Results

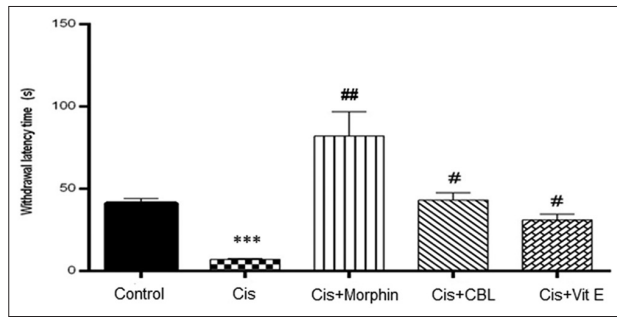
### CBL treatment attenuated cisplatin-induced cold hyperalgesia

As shown in Figure 1, a single dose injection of Cis significantly ( $p < 0.01$ ) decreased withdrawal latency time in the cold plate test

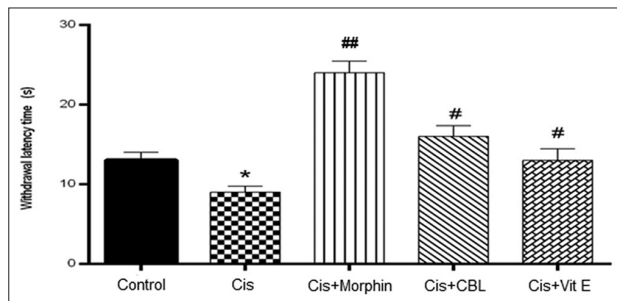
when compared to the control group. However, injection of CBL and Vit E markedly ( $p<0.05$ ) ameliorated cold plate hyperalgesia compared to the Cis group. Moreover, a single dose injection of 10 mg/kg morphine significantly ( $p<0.05$ ) increased withdrawal latency time in the cold plate test. The injection method (i.v. vs. i.p.) did not produce any significant effect in comparison to the control group; therefore these results are not shown. (Fig. 1).

**CBL attenuated cisplatin-induced heat hyperalgesia**

Cis induced a significant reduction in the latency response time to acute heat stimuli compared to the control group ( $p<0.05$ , Figure 2). An increased reaction time in the hot plate test was observed in the Cis + CBL, Cis + Vit E, and Cis + Morphine groups compared with the Cis group ( $p<0.05$ ). The injection method (i.v. vs. i.p.) did not change the latency time in comparison to the control group; therefore these results are not presented.



**Figure 1.** Cold hyperalgesia in the BALB/c mice models of cisplatin neuropathic pain. Data are presented as mean  $\pm$  S.E.M (n = 6). \*\*\* $p<0.001$  compared to the control group and # $p<0.05$ , ## $p<0.01$  compared to the Cis group. Cisplatin (Cis); Cerebrolysin (CBL); vitamin E (Vit E).



**Figure 2.** Hot plate test of cisplatin-induced neuropathic pain models in BALB/c mice. Data are presented as mean  $\pm$  S.E.M (n = 6). \* $p<0.05$  compared to the control group and # $p<0.05$ , ## $p<0.01$  compared to the Cis group. Cisplatin (Cis); Cerebrolysin (CBL); Vitamin E (Vit E).

**CBL attenuated the late phase of formalin pain**

Formalin injection into the hind paw of mice in the control group induced a significant ( $p<0.05$ ) pain response within the first 5-minute interval (acute phase) and over the 10-50 minute interval. As presented in Table 1, the nociceptive-inducing effect of formalin significantly increased (both in the acute and late phase) in the Cis group compared to the control group ( $p<0.05$ ). In addition, formalin-evoked pain responses in the early phase were reduced only in the Cis + Morphine group and this effect was observed in neither the Cis + CBL nor the Cis + Vit E groups. In contrast to the early phase, formalin-inducing nociceptive behaviors were significantly reduced in the Cis + CBL group ( $p<0.05$ ). The duration of the late phase of formalin test was significantly reduced in the Cis + CBL, Cis + Morphine and Cis + Vit E groups.

**Discussion**

Platinum-based cancer chemotherapy has been used for decades and despite its widespread clinical use, adverse side effects, including peripheral neuropathy, are the main dose-limiting troublesome factors. As a crucial neurotoxic side effect, CIPN mechanisms are poorly understood [11]. Several experimental assessments have been developed to evaluate CIPN incidence and test compounds which could be useful in the treatment of this complication [3, 33].

In the present study, results showed that a single injection of Cis was able to produce hyperalgesia and significantly decrease the withdrawal latency threshold, which was attenuated by CBL treatment. To our knowledge, this is the first report showing that CBL is protective against Cis-induced hyperalgesia in different behavioral tests in mice.

CBL is a combination of neurotrophic factors and has been reported to improve different neurological conditions such as stroke, dementia, diabetic neuropathic pain, and spinal cord injury [16, 17, 21, 22]. It has been shown that CBL alleviates mechanical and thermal hypersensitivity induced by nitroglycerin [23]. A previous study showed that Cis induced a pro-inflammatory response and played a role in neuropathic induction [34]. In the current study, CBL significantly attenuates the late phase of formalin pain, possibly through the inhibition of pro-inflammatory cytokines released by immune cells at the inflammation site.

Several mechanisms could be responsible for the neuroprotective property of CBL. The antinociceptive effect of CBL is in part mediated through reduction of oxidative stress, inflammatory cascade, and excitotoxic and apoptotic

**Table 1:** Licking and biting time course of the formalin-injected paw within 5-min-long intervals.

Groups/intervals	0-5	5-10	10-15	15-20	20-25	25-30	30-35	35-40	40-45	45-50	50-55	55-60
Control	50.4 $\pm$ 3.2	9.8 $\pm$ 2.3	12 $\pm$ 3	35.2 $\pm$ 2	42 $\pm$ 1	28.6 $\pm$ 1.5	24.4 $\pm$ 2.7	27.2 $\pm$ 2.1	25 $\pm$ 3.4	30 $\pm$ 2.9	33 $\pm$ 2.6	25 $\pm$ 1.8
Cis	83.2 $\pm$ 3.2*	8 $\pm$ 4.4	30.8 $\pm$ 3.2*	59.2 $\pm$ 3.2*	62.6 $\pm$ 1.1*	80.8 $\pm$ 3.2*	70 $\pm$ 2.4*	55 $\pm$ 2.2*	42 $\pm$ 4.3*	39 $\pm$ 3.4*	41 $\pm$ 2.3*	46.4 $\pm$ 4.1*
Cis & Morphine	19 $\pm$ 1.2#	0.6 $\pm$ 0.1#	0.8 $\pm$ 0.1#	8 $\pm$ 0.1#	12 $\pm$ 0.5#	15.2 $\pm$ 0.1#	13 $\pm$ 0.2#	27 $\pm$ 0.8#	28.6 $\pm$ 0.9#	30 $\pm$ 0.5#	37.6 $\pm$ 2.1	40 $\pm$ 2.3
Cis & CBL	86 $\pm$ 4.2	11.4 $\pm$ 0.4	13.7 $\pm$ 0.1#	35.2 $\pm$ 1.2#	38.2 $\pm$ 2.6#	39.7 $\pm$ 0.3#	31.2 $\pm$ 0.2#	36.2 $\pm$ 0.1#	25.2 $\pm$ 0.4#	30.5 $\pm$ 0.9#	35.7 $\pm$ 2.1#	42.5 $\pm$ 1.4
Cis & Vit E	79.2 $\pm$ 3.2	9.2 $\pm$ 0.4	11.2 $\pm$ 0.1#	39.8 $\pm$ 0.2#	53 $\pm$ 2.6	58 $\pm$ 2.3#	51 $\pm$ 3.2	32.2 $\pm$ 0.1	30 $\pm$ 0.4	32.8 $\pm$ 0.9	40 $\pm$ 2.1	44.4 $\pm$ 1.4

Data are presented as mean $\pm$ S.E.M (n=6). \* $p<0.05$  compared to the control group and # $p<0.05$  compared to the formalin group. Cisplatin (Cis); Cerebrolysin (CBL); Vitamin E (Vit E).

cell death [22]. One of the underlying mechanisms involved in platinum agents' peripheral neurotoxicity is mediated through increasing the oxidative stress burden [13, 35]. Antioxidant agents are involved in pain control possibly through the prevention of the negative impact of reactive oxygen species (ROS) on nociception. Previous studies have shown that compounds with anti-oxidant activity can ameliorate cisplatin-induced neuropathic pain [13, 35]. It has also been reported that systematic injections of vitamin E, a common antioxidant, produce an analgesic effect in neuropathic rats [6]. Similarly, this study found that administration of 5 g/kg of Vit E for 4 days significantly reduced hyperalgesia induced by Cis. Georgy et al. showed that CBL decreased nitric oxide formation and increased levels of pyruvate which scavenged  $H_2O_2$ , resulting in oxidative stress reduction in type III diabetes [15]. Therefore, it appears that CBL may reduce neuropathic pain through its antioxidant property.

Another mechanism which may contribute to CBL effects is its anti-inflammatory property. Pro-inflammatory cytokines have an important role in the development of inflammatory hyperalgesia, while anti-inflammatory cytokines inhibit pain responses [36]. Notably, TNF- $\alpha$  acts as an intermediary in free radical production; hence its inhibition can attenuate oxidative stress injuries [37]. According to a study conducted by Alvarez et al., CBL exerted anti-inflammatory effects through reduction of TNF- $\alpha$  levels [38]. Similar to previous reports, our results showed that Cis intensified both phases of formalin test responses, especially the duration of licking and biting time in the late phase. It is known that the formalin test induces two main phases, an early phase with activation of C fiber and a delayed phase with an inflammatory response in peripheral tissues that provokes spinal nociceptive neuronal mechanisms [39-41]. Therefore, we suggest that the effectiveness of CBL in reducing nociception in the late phase of formalin test may arise from its anti-inflammatory property.

## Conclusion

Although morphine is a common choice in the treatment of both severe post-operative pain and cancer pain, its clinical use is restricted because of side effects such as addiction. In the present study, results showed that CBL treatment produced a significant antinociceptive action. Thus, the antinociceptive effect of CBL may provide a potential new focus for developing novel analgesic drugs without the liability of possible abuse and opioid dependency. However, more studies are indicated to examine the exact mechanisms of pain-reducing effects of CBL.

## Conflicts of Interest

The authors report no conflicts of interest.

## References

- Dworkin RH, O'connor AB, Backonja M, Farrar JT, Finnerup NB, Jensen TS, et al. Pharmacologic management of neuropathic pain: evidence-based recommendations. *Pain*. 2007; 132(3):237-51.
- Edwards JL, Vincent AM, Cheng HT, Feldman EL: Diabetic neuropathy: mechanisms to management. *Pharmacology & therapeutics*. 2008; 120(1):1-34.
- Wolf S, Barton D, Kottschade L, Grothey A, Loprinzi C. Chemotherapy-induced peripheral neuropathy: prevention and treatment strategies. *European Journal of Cancer*. 2008; 44(11):1507-15.
- Kayatnouri MH, Namvaran-Abasabad A. Antinociceptive effect of *Matricaria chamomilla* on vincristine-induced peripheral neuropathy in mice. *African Journal of Pharmacy and Pharmacology*. 2012; 6(1):24-9.
- Namvaran-Abasabad A, Kayatnouri MH, Tavakoli, F. Effect of *Salvia officinalis* Hydroalcoholic Extract on Vincristine-induced Neuropathy in Mice. *Chinese Journal of Natural Medicines*. 2011; 9(5):354-8.
- Kim H, Kim JH, Gao X, Zhou J-L, Lee I, Chung K, et al. Analgesic effect of vitamin E is mediated by reducing central sensitization in neuropathic pain. *Pain*. 2006; 122(1):53-62.
- Postma T, Heimans J. Grading of chemotherapy-induced peripheral neuropathy. *Annals of Oncology*. 2000; 11(5):509-13.
- Dunlap B, Paice JA. Chemotherapy-induced peripheral neuropathy: a need for standardization in measurement. *J Support Oncol*. 2006; 4(8):398-9.
- Florea A-M, Büsselberg D. Cisplatin as an anti-tumor drug: cellular mechanisms of activity, drug resistance and induced side effects. *Cancers*. 2011; 3(1):1351-71.
- Maestri A, De Pasquale, Ceratti A, Cundari S, Zanna C, Cortesi E, Crinò L. A pilot study on the effect of acetyl-L-carnitine in paclitaxel-and cisplatin-induced peripheral neuropathy. *Tumori*. 2005; 91(2):135-8.
- Namvaran-Abbas-Abad A, Tavakkoli F. Antinociceptive effect of salvia extract on cisplatin-induced hyperalgesia in mice. *Neurophysiology*. 2012; 43(6):452-8.
- Ta LE, Low PA, Windebank AJ. Mice with cisplatin and oxaliplatin-induced painful neuropathy develop distinct early responses to thermal stimuli. *Mol Pain*. 2009; 5:9.
- Abad ANA, Nouri MHK, Gharjanie A, F T. Effect of *Matricaria chamomilla* Hydroalcoholic Extract on Cisplatin-induced Neuropathy in Mice. *Chinese Journal of Natural Medicines*. 2011; 9(2):126-31.
- Rao RD, Flynn PJ, Sloan JA, Wong GY, Novotny P, Johnson DB, et al. Efficacy of lamotrigine in the management of chemotherapy-induced peripheral neuropathy. *Cancer*. 2008; 112(12):2802-8.
- Georgy GS, Nassar NN, Mansour HA, Abdallah DM. Cerebrolysin ameliorates cognitive deficits in type III diabetic rats. *PloS one*. 2013;8(6):e64847.
- Kumaran Menon P, Fior Muresanu D, Sharma A, Mossler H, Shanker Sharma H. Cerebrolysin, a mixture of neurotrophic factors induces marked neuroprotection in spinal cord injury following intoxication of engineered nanoparticles from metals. *CNS & Neurological Disorders-Drug Targets (Formerly Current Drug Targets-CNS & Neurological Disorders)*. 2012; 11(1):40-9.
- Ruether E, Ritter R, Apecechea M, Freytag S, Gmeinbauer R, Windisch M. Sustained improvements in patients with dementia of Alzheimer's type (DAT) 6 months after termination of Cerebrolysin therapy. *Journal of neural transmission*. 2000; 107(7):815-29.
- Zhang C, Chopp M, Cui Y, Wang L, Zhang R, Zhang L, et al. Cerebrolysin enhances neurogenesis in the ischemic brain and

- improves functional outcome after stroke. *Journal of neuroscience research*. 2010; 88(15):3275-81
19. Rockenstein E, Mante M, Adame A, Crews L, Moessler H, Masliah E. Effects of Cerebrolysin™ on neurogenesis in an APP transgenic model of Alzheimer's disease. *Acta neuropathologica*. 2007; 113(3):265-75.
  20. Zhang Y, Chopp M, Meng Y, Zhang ZG, Doppler E, Winter S, et al. Cerebrolysin improves cognitive performance in rats after mild traumatic brain injury. *Journal of neurosurgery*. 2015; 122(4):843-55.
  21. Biesenbach G, Grafinger P, Eichbauer-Sturm G, Zazgornik J. [Cerebrolysin in treatment of painful diabetic neuropathy]. *Wiener medizinische Wochenschrift (1946)*. 1996; 147(3):63-6.
  22. Masliah E, Diez-Tejedor E. The pharmacology of neurotrophic treatment with Cerebrolysin: brain protection and repair to counteract pathologies of acute and chronic neurological disorders. *Drugs of today (Barcelona, Spain)*. 2012; 48:3-24.
  23. Mahmoudi J, Mohaddes G, Erfani M, Sadigh-Eteghad S, Karimi P, Rajabi M, et al. Cerebrolysin attenuates hyperalgesia, photophobia, and neuroinflammation in a nitroglycerin-induced migraine model in rats. *Brain research bulletin*. 2018; 140:197-204.
  24. Boucher TJ, McMahon SB. Neurotrophic factors and neuropathic pain. *Current Opinion in Pharmacology*. 2001; 1(1):66-72
  25. Abdel-Salam OM, Baiuomy AR, Arbid MS. Studies on the anti-inflammatory effect of fluoxetine in the rat. *Pharmacol Res*. 2004;49(2):119-31.
  26. Kim HK, Kim JH, Gao X, Zhou JL, Lee I, Chung K, et al. Analgesic effect of vitamin E is mediated by reducing central sensitization in neuropathic pain. *Pain*. 2006; 122(1-2):53-62.
  27. Vahidi-Eyrisofla N, Yazdian MR, Mahmoudi J. Therapeutic and prophylactic effects of chronic cerebrolysin regimen on the motor disorders of hyperglycemic rats. *Medical Journal of Tabriz University of Medical Sciences and Health Services*. 2015; In press.
  28. Zangiabadi N, Mohtashami H, Shabani M, Jafari M. Neuroprotective Effect of Cerebrolysin on Diabetic Neuropathy: A Study on Male Rats. *journal of diabetes & metabolism*. 2014; 5(4):357.
  29. Zhang C, Chopp M, Cui Y, Wang L, Zhang R, Zhang L, et al. Cerebrolysin enhances neurogenesis in the ischemic brain and improves functional outcome after stroke. *J Neurosci Res*. 2010; 88(15):3275-81.
  30. Allchorne AJ, Broom DC, Woolf CJ. Detection of cold pain, cold allodynia and cold hyperalgesia in freely behaving rats. *Mol Pain*. 2005; 1:36
  31. Christoph T, Kogel B, Schiene K, Meen M, De Vry J, Friderichs E. Broad analgesic profile of buprenorphine in rodent models of acute and chronic pain. *Eur J Pharmacol*. 2005 Jan 10; 507(1-3):87-98.
  32. Nayebi AM, Nazemiyeh H, Omidbakhsh R, Çobanoğlu S. Analgesic effect of the methanol extract of *Erica arborea* (L.) in mice using formalin test. *DARU Journal of Pharmaceutical Sciences*. 2015; 16(4):229-32.
  33. Han Y, Smith MT. Pathobiology of cancer chemotherapy-induced peripheral neuropathy (CIPN). *Front Pharmacol*. 2013;4:156.
  34. Kim S-J, Lim J-Y, Lee JN, Choe S-K, Kim Y-I, Song SR, et al. Activation of  $\beta$ -catenin by inhibitors of glycogen synthase kinase-3 ameliorates cisplatin-induced cytotoxicity and pro-inflammatory cytokine expression in HEI-OC1 cells. *Toxicology*. 2014; 320:74-82.
  35. Namvaran Abbas Abad A, Khayate Nouri MH, Tavakkoli F. Effect of *Salvia officinalis* Hydroalcoholic Extract on Vincristine-induced Neuropathy in Mice. *Chinese Journal of Natural Medicines*. 2011; 9(5):354-8.
  36. Azizzadeh F, Mahmoodi J, Sadigh-Eteghad S, Farajdokht F, Mohaddes G. Ghrelin exerts analgesic effects through modulation of IL-10 and TGF- $\beta$  levels in a rat model of inflammatory pain. *Iranian biomedical journal*. 2017;21(2):114.
  37. Ingaramo PI, Ronco MT, Frances DE, Monti JA, Pisani GB, Ceballos MP, et al.: Tumor necrosis factor alpha pathways develops liver apoptosis in type 1 diabetes mellitus. *Molecular immunology*. 2011; 48(12-13):1397-407.
  38. Alvarez XA, Sampedro C, Cacabelos R, Linares C, Aleixandre M, Garcia-Fantini M, et al. Reduced TNF- $\alpha$  and increased IGF-I levels in the serum of Alzheimer's disease patients treated with the neurotrophic agent Cerebrolysin. *The International Journal of Neuropsychopharmacology*. 2009; 12(07):867-72.
  39. Shibata M, Ohkubo T, Takahashi H, Inoki R. Modified formalin test: characteristic biphasic pain response. *Pain*. 1989; 38(3):347-52.
  40. Sul JK, Deangelis LM. Neurologic complications of cancer chemotherapy. *Seminars in oncology*. 2006 Jun; 33(3):324-32.
  41. Joseph EK, Levine JD. Mitochondrial electron transport in models of neuropathic and inflammatory pain. *Pain*. 2006; 121(1-2):105-14