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Research Paper

Preclinical evidence for the anxiolytic- and antidepressant-like effects of citicoline and imipramine in the sciatic nerve-ligated mice

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ABSTRACT

Background: Neuropathic pain is a usual condition followed by nerve injury. Experimental neuropathy is linked with delayed behavioral variations correlated to anxiety and depression behaviors. Imipramine is a tricyclic antidepressant that can diminish anxiety- and depressive-like behaviors. Also, citicoline as a dietary supplement has antidepressant and anxiolytic effects.

Methods: We sought to investigate citicoline's effect on anxiety-like (by elevated plus-maze (EPM)) and depression-like (by tail suspension test (TST)) responses as well as its potential to increase imipramine antidepressant properties in nerve-ligated mice.

Results: The results showed that induction of neuropathic pain through sciatic nerve ligation caused anxious- and depressant-like behaviors in male mice. On the other hand, intraperitoneal (i.p.) injections of moderate (50 mg/kg) and high (100 mg/kg) doses of citicoline and high dose of imipramine (5 mg/kg) significantly reduced anxiety- and depression-like behaviors induced by sciatic nerve ligation in male mice. Additionally, a low (25 mg/kg) dose of citicoline potentiated the anxiolytic- and antidepressant-like effects of different doses of imipramine when they co-injected in nerve-ligated mice. Isobolographic analysis indicated an additive effect of imipramine and citicoline on the occurrence of anxiolytic- and antidepressant-like behaviors in nerve-ligated mice. Our results showed that citicoline alone reduces anxiety- and depression-like behaviors. Furthermore, when co-administered with imipramine, citicoline potentiates imipramine effects.

Conclusions: Injection of citicoline (as a dietary supplement) along with imipramine improved the effectiveness of imipramine for the management of anxiety- and depressive-like responses in nerve-ligated mice.

1. Introduction

Neuropathic pain is considered a model of chronic pain that elicits loss or atypical activity of the central or peripheral nerve systems (Benbouzid et al., 2008; Hashemzaei et al., 2017). It can change the patient's quality of life through interference with emotional well-being (Galer et al., 2000). Clinical research has indicated a link between chronic pain, mood disorders, and anxiety phenotype (Dersh et al., 2002; Narita et al., 2006; Suzuki et al., 2007). Because neuropathic pain is multifactorial, different neuropathic pain animal models were used, as much of the current knowledge comes from investigations with rats and mice (Sant'Anna et al., 2016; Sousa et al., 2016; Suzuki et al., 2007).

Imipramine is a tricyclic antidepressant. It is most frequently administrated for treating depressive disease (Forlenza et al., 2000; Ramirez & Sheridan, 2016). It can decrease anxiety- and depressive-like behaviors (Paolo & Galistu, 2019; Ramirez & Sheridan, 2016; Yanagida et al., 2016). The mechanism of action of tricyclic antidepressants such as imipramine for inducing therapeutic effects is related to the monoaminergic system. These substances directly inhibiting the neurotransmitter transporters prevent the reuptake of serotonin, dopamine, and

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norepinephrine (Zhou et al., 2007). The transporters for serotonin, dopamine, and norepinephrine within the presynaptic neuron restrict the signal transmission of neurons (Iversen, 2006). Compounds used to inhibit these transporters have been administrated effectively for the treatment of anxiety and depression (Ramirez & Sheridan, 2016).

Citicoline is an endogenous substance (Secades, 2016, 2021). Citicoline is generally accessible as a food supplement but in several countries, it is used as a drug. In hydrolysis and dephosphorylation processes, citicoline changed to cytidine and choline. Cytidine and choline are substances for the synthesis of phosphatidylcholine and citicoline in neurons (Iulia et al., 2017; Jasielski et al., 2020; Paulose et al., 2017; Roohi-Azizi et al., 2017). Citicoline has negligible harmful effects. It is quickly metabolized. Metabolic products are removed as carbon dioxide. The safety of citicoline has been frequently demonstrated in animal investigations (Grieb, 2014). It has complete neuroprotective effects and useful effects on neurodegenerative diseases (Alvarez-Sabin & Roman, 2013; Cho & Kim, 2009; Conant & Schauss, 2004; Jasielski et al., 2020). Citicoline also modulates the quantity of neurotransmitters in the synapses. It increases the level of acetylcholine, dopamine, serotonin, and norepinephrine in the CNS (Secades, 2016). These neurotransmitters also have neuroprotective effects (Jasielski et al., 2020). Also, citicoline has antidepressant and anxiolytic properties (Abdolmaleki et al., 2016; Arcadi et al., 2021; Brown & Gabrielson, 2012; Brown et al., 2015; Carlezon et al., 2002; Nejati et al., 2020; Roohi-Azizi et al., 2017). Imipramine and citicoline play a role in the control of anxiety and depression processes as well as involvement in the control of neurotransmitter releases such as acetylcholine, dopamine, serotonin, and norepinephrine (Paolo & Galistu, 2019; Zhou et al., 2007), this study was designed to assess citicoline effect on anxiety- and depression-like effects as well as its potential to rise imipramine antidepressant characteristic in nerve-ligated mice by elevated plus-maze (EPM) device and tail suspension test (TST).

2. Materials and methods

2.1. Animals

Adult NMRI male albino mice (6–8 weeks old and 20–25 g weight) were utilized in this investigation. Animals were maintained in an animal house with a 12 h light: dark cycle and constant temperature (23 ± 1 °C). Mice were housed 4–6 per cage in Plexiglas cages with free access to food and water. Eight mice were used for each experimental group. Tests were carried out according to the guidelines of care and use of laboratory animals which were confirmed by the Animal Research Ethics Committee of the Tehran University of Medical Sciences (NIH publications No. 80-23).

2.2. Drugs

Citicoline sodium (Minoo, Tehran, Iran) and imipramine (Ciba-Geigy, Switzerland) were utilized in this experiment. All doses of citicoline (25, 50, 100 mg/kg) and imipramine (1.25, 2.5, and 5 mg/kg) were dissolved in saline 0.9 % and were administrated intraperitoneally (i.p.) with a volume of 10 ml/kg. The control mice received an injection of saline 0.9 % (10 ml/kg). The rationale for choosing the range of dug dosages were determined based on pilot studies and previous studies (Khakpai et al., 2021).

2.3. Surgical procedures

Animal anesthesia was done with ketamine (50 mg/kg; i.p.) and xylazine (4 mg/kg; i.p.) mixture. Based on our previous investigation, unilateral peripheral neuropathy was elicited on the right hind limb (M. Zarrindast et al., 2000). The mouse's right sciatic nerve was uncovered. A 2 mm long nerve part was dissected. Then, one ligature by suture thread was used near the dissected sciatic nerve. In sham-operated mice, the above technique was done except for the dissection of the sciatic nerve.

2.4. Elevated Plus-Maze (EPM)

The elevated plus-maze (EPM) device has been broadly used to study novel anxiolytic substances and to evaluate the neurochemical and physiological base of anxiety phenotype (Matsuzawa-Yanagida et al., 2008). Hence, we used the EPM to examine anxiety-like responses. The EPM device is made of two opposing open (30 × 5 cm^2) and two opposing closed arms (30 × 5 × 15 cm^3) combined through a common central platform (5 × 5 cm^2). This apparatus was located 40 cm above the floor. An arm entry or exit was determined as all paws in and out of each arm. Data were measured as follows: %OAT (the ratio of time spent in the open arms to the total time spent in all arms × 100); %OAE (the ratio of entries in the open arms to total entries in all arms × 100). Closed arm entries were considered a pure index of locomotor activity.

2.5. Tail suspension test (TST)

To examine depression-like behavior, we used the tail suspension test (TST). In the TST, the whole time of immobility provoked via tail suspension was calculated. Animals lonely were suspended 50 cm above the floor through adhesive tape positioned about 1 cm from the tip of the mouse tail. The TST was carried out for 6 min in which 2 min were considered for habituation and in the residual 4 min, immobility time was calculated.

2.6. Study design

This study consisted of four experiments.

In experiment 1, three groups were used as follows: i) control group (injection of saline (10 ml/kg) to naïve mice), sham group (injection of saline (10 ml/kg) to mice that underwent surgery but the sciatic nerve was not dissected) and surgical neuropathy (injection of saline (10 ml/kg) to mice that were operated and the sciatic nerve was dissected). This experiment investigated the effects of injections, anesthetics, and neuropathy surgery at different times before and after nerve ligation on % Oat%OAE, and locomotor activity in the EPM and immobility time of the TST. Based on this, in the next experiments, the neuropathy surgery group was considered the control group to compare the effects of the drugs.

Experiment 2 examined the influence of i.p. administration of saline (10 ml/kg) and different dosages of citicoline at low (25 mg/kg), moderate (50 mg/kg), and high (100 mg/kg) doses on %Oat%OAE, and locomotor activity in the EPM and immobility time of the TST.

Experiment 3 investigated the influence of alone i.p. injection of saline (10 ml/kg) or different dosages of imipramine at low (1.25 mg/kg), moderate (2.5 mg/kg), and high (5 mg/kg) doses, and co-injection of these doses of imipramine plus a low dose of citicoline (25 mg/kg) on the performance of mice in EPM and TST.

Experiment 4 assessed the influence of co-treatments of imipramine 2.5 mg/kg + citicoline 50 mg/kg, imipramine 1.25 mg/kg + citicoline 25 mg/kg, and imipramine 0.625 mg/kg + citicoline 12.5 mg/kg on anxiety- and depression-related effects. 10 min after drug administration, EPM, and TST were carried out in separate groups. Table 1 and Fig. 1 describe the experimental groups.

2.7. Statistical analysis

Results were indicated as the mean ± standard error of the mean (SEM). After checking the homogeneity of variances, data were evaluated with one-way and two-way ANOVA followed by Tukey's multiple comparisons. The level of statistical significance was considered as $P < 0.05$.

It should be noted that isobolographic analysis was performed to

Table 1
Describes the experimental groups.

Figure	Panel	Drug treatments (i.p.)	Effect on anxiety	Effect on depression
1	A	Control, sham, and sciatic nerve ligated (saline, 10 ml/kg)	Anxiogenic	–
	B	Control, sham, and sciatic nerve ligated (saline, 10 ml/kg)	–	–
	C	Control, sham, and sciatic nerve ligated (saline, 10 ml/kg)	–	–
	D	Control, sham, and sciatic nerve ligated (saline, 10 ml/kg)	–	Depressant
2	A	Saline (10 ml/kg), citicoline (25, 50, and 100 mg/kg)	Anxiolytic	–
	B	Saline (10 ml/kg), citicoline (25, 50, and 100 mg/kg)	–	–
	C	Saline (10 ml/kg), citicoline (25, 50, and 100 mg/kg)	–	–
	D	Saline (10 ml/kg), citicoline (25, 50, and 100 mg/kg	–	Antidepressant
3	A(Left panel)	Saline (10 ml/kg), imipramine (1.25, 2.5, and 5 mg/kg)	Anxiolytic	–
	A(Right panel)	Saline (10 ml/kg), imipramine (1.25, 2.5, and 5 mg/kg) + citicoline (25 mg/kg)	Anxiolytic	–
	B(Left panel)	Saline (10 ml/kg), imipramine (1.25, 2.5, and 5 mg/kg)	–	–
	B(Right panel)	Saline (10 ml/kg), imipramine (1.25, 2.5, and 5 mg/kg) + citicoline (25 mg/kg)	Anxiolytic	–
	C(Left panel)	Saline (10 ml/kg), imipramine (1.25, 2.5, and 5 mg/kg)	–	–
	C(Right panel)	Saline (10 ml/kg), imipramine (1.25, 2.5, and 5 mg/kg) + citicoline (25 mg/kg)	–	–
	D(Left panel)	Saline (10 ml/kg), imipramine (1.25, 2.5, and 5 mg/kg)	–	Antidepressant
	D(Right panel)	Saline (10 ml/kg), imipramine (1.25, 2.5, and 5 mg/kg) + citicoline (25 mg/kg)		Antidepressant
4	A	Imipramine 2.5 mg/kg + citicoline 50 mg/kg Imipramine 1.25 mg/kg + citicoline 25 mg/kg Imipramine 0.625 mg/kg + citicoline 12.5 mg/kg	Additive anxiolytic	–
	B	Imipramine 2.5 mg/kg + citicoline 50 mg/kg Imipramine 1.25 mg/kg + citicoline 25 mg/kg Imipramine 0.625 mg/kg + citicoline 12.5 mg/kg	–	Additive antidepressant

determine the cross-talk after the treatment with two drugs. The ED50 of each drug (imipramine 2.5 mg/ kg and citicoline 50 mg/kg) was tested by linear regression analysis. Treatment with two drugs was carried out in a constant dosage ratio upon the ED50. For drug co-treatment, the theoretic ED50 is imipramine ED50/2 + citicoline ED50/2. Moreover, the experimental result of drug co-treatment from the fixed ratio considered was examined with the regression analysis, after that the experimental ED50 result of the drug co-treatment was measured (% 50 %OAT of the EPM and %50 immobility time of the TST). One-sample t-test was done to evaluate the statistical significance difference between the theoretical ED50 and experimental ED50 of the drug co-treatment. Once the experimental ED50 significantly was lower than the theoretical ED50 a synergistic interaction between imipramine and citicoline was determined; however, there was not any difference among them, displaying additive interaction rather than the synergistic response (Saadati et al., 2022). Variances with $P < 0.05$ among the test groups at each point were considered statistically significant.

3. Results

3.1. The effect of sciatic nerve ligation on anxiety- and depression-like responses

The influence of sciatic nerve ligation on anxiety- and depression-like symptoms was assessed by performing a primary test consisting of three groups; the control, sham, and sciatic nerve ligated groups (Fig. 2). These three groups received saline (10 ml/kg). One-way ANOVA and post hoc analysis showed that sciatic nerve ligation reduced %OAT [$F(2, 21) = 5.616$, $p = 0.011$, Mean ± SEM = 14.541 ± 7.961, Fig. 2A] and % OAE [$F(2, 21) = 4.710$, $p = 0.020$, Mean ± SEM = 20.666 ± 10.129, Fig. 2B], but enhanced immobility time of the TST [$F(2, 21) = 5.149$, $P = 0.017$, Mean ± SEM = 118.833 ± 43.356, Fig. 2D] compared to the control and sham groups. Locomotor activity [$F(2, 21) = 0.379$, $p = 0.689$, Mean ± SEM = 9.958 ± 1.706, Fig. 2C] did not change.

3.2. The effect of citicoline on anxiety- and depression-like responses

Fig. 3 indicated the influence of i.p. administration of different dosages of citicoline at low (25 mg/kg), moderate (50 mg/kg), and high (100 mg/kg) doses on anxiety- and depression-like responses using EPM and TST in nerve-ligated mice. According to one-way ANOVA and post hoc analysis, injection of citicoline increased %OAT [$F(3, 28) = 9.301$, $p = 0.000$, Mean ± SEM = 17.437 ± 11.138, Fig. 3A] and %OAE [$F(3, 28 = 3.652$, $p = 0.024$, Mean ± SEM = 20.750 ± 10.344, Fig. 3B], but decreased immobility time of the TST [$F(3, 28) = 12.002$, $P = 0.000$, Mean ± SEM = 105.875 ± 51.795, Fig. 3D] at the moderate (50 mg/kg) and high (100 mg/kg) doses, in comparison to the saline group. These dosages of citicoline had no significant effect on locomotor activity [$F(3, 28) = 0.602$, $p = 0.519$, Mean ± SEM = 9.781 ± 1.913, Fig. 3C].

3.3. The effect of imipramine alone or along with citicoline on anxiety- and depression-like responses

The effects of i.p. injection of different dosages of imipramine at low (1.25 mg/kg), moderate (2.5 mg/kg), and high (5 mg/kg) doses and co-treatment of these dosages plus a low dose of citicoline (25 mg/kg) on anxiety- and depression-like responses in nerve-ligated mice are presented in Fig. 4. According to one-way ANOVA and post hoc analysis, high dose of imipramine (5 mg/kg; i.p.) raised %OAT [$F(3, 28) = 3.000$, $p = 0.031$, Mean ± SEM = 13.531 ± 4.071, Fig. 4A, left panel] but low

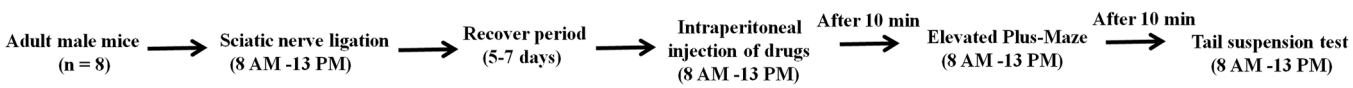

Fig. 1. The timing of the experimental procedures/steps.

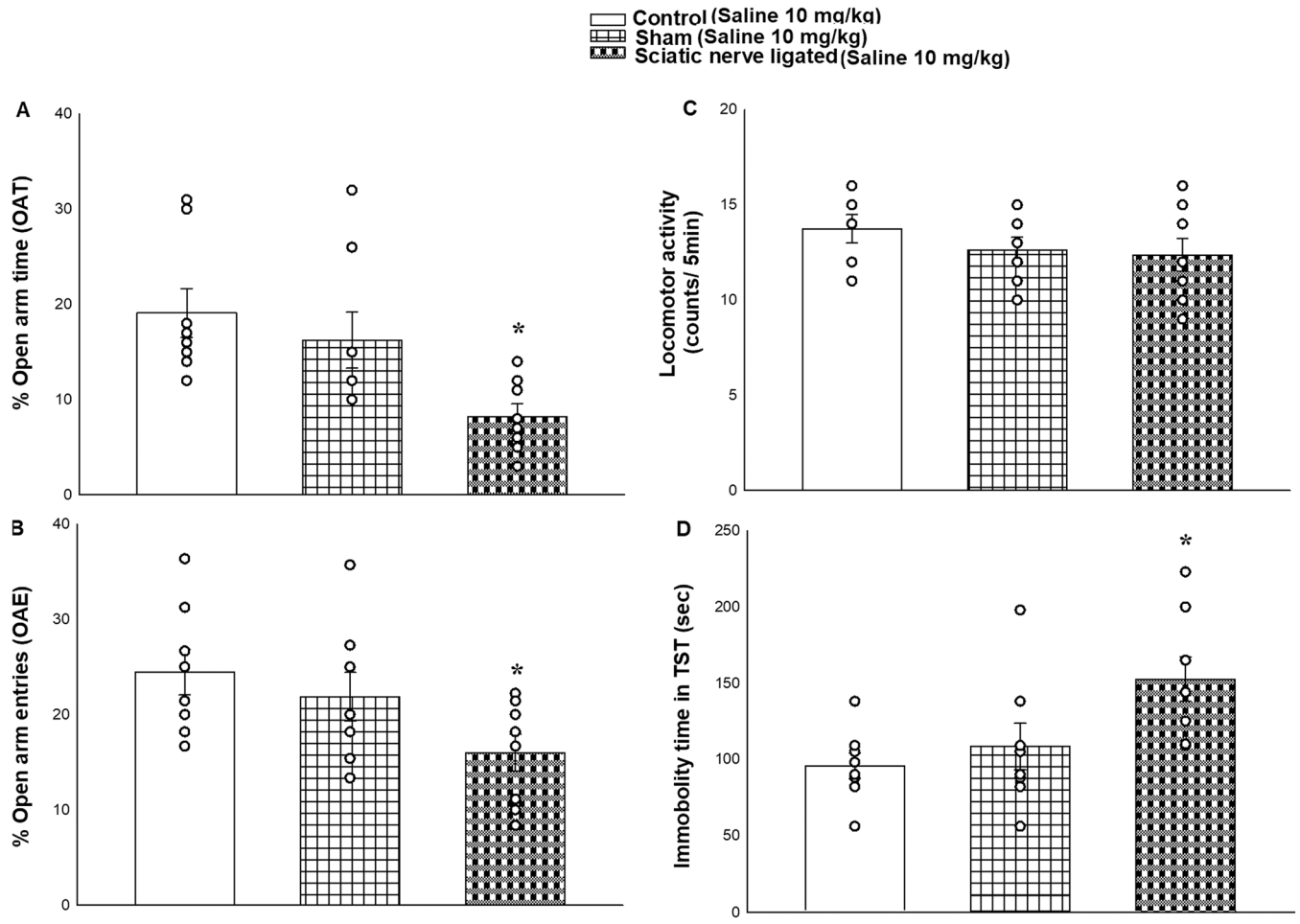

Fig. 2. The effect of sciatic nerve ligation on anxiety- and depression-like responses on %OAT (A), %OAE (B), locomotor activity (c) in the EPM as well as immobility time of the TST (D) in nerve-ligated mice. Data indicated as mean ± S.E.M. (n = 8). *P < 0.05 compared with the control group.

(2.5 mg/kg) and moderate (5 mg/kg) dosages of imipramine reduced immobility time of the TST [F (3, 28) = 16.542, P =0.000, Mean ± SEM = 92.750 ± 31.214, Fig. 4D, left panel] in comparison to the saline group. Nevertheless, these injections had no significant influence on % OAE [F (3, 28) = 0.888, p = 0.462, Mean ± SEM = 10.718 ± 1.888, Fig. 4B, left panel] and locomotor activity [F (3, 28) = 0.053, p = 0.995, Mean ± SEM = 7.843 ± 2.908, Fig. 4C, left panel].

According to two-way ANOVA, there was no significant interaction between the effect of co-administration of a low dose of citicoline (25 mg/kg) and different doses of imipramine on %OAT [treatment effect: F (1, 56) = 81.403, P = 0.000, dose effect: F (3, 56) = 5.051, P = 0.001, treatment–dose interaction: F (3, 56) = 0.491, P = 0.762; Mean ± SEM = 29.406 ± 8.533; Fig. 4A; right panel], %OAE [treatment effect: F (1, 56) = 80.673, P = 0.000, dose effect: F (3, 56) = 1.365, P = 0.255, treatment–dose interaction: F (3, 56) = 0.078, P = 0.989; Mean ± SEM = 20.765 ± 10.455; Fig. 4B; right panel], and locomotor activity of the EPM [treatment effect: F (1, 56) = 1.744, P = 0.682, dose effect: F (3, 56) = 0.054, P = 0.994, treatment–dose interaction: F (3, 56) = 0.147, P = 0.9624; Mean ± SEM = 8.734 ± 2.829; Fig. 4C; right panel], and immobility time of the TST [treatment effect: F (1, 56) = 45.733, P = 0.000, dose effect: F (3, 56) = 23.791, P = 0.000, treatment–dose interaction: F (3, 56) = 0.234, P = 0.918; Mean ± SEM = 75.031 ± 36.287; Fig. 4D; right panel]. Post hoc analysis revealed that co-treatment of different doses of imipramine (1.25, 2.5, and 5 mg/kg) and a low dose of citicoline (25 mg/kg) significantly increased %OAT and %OAE in the EPM but reduced immobility time in the TST.

3.4. The additive influence among imipramine and citicoline on anxiolytic- and antidepressant-like responses

The theoretical additive line revealed that at all points, co-treatment of imipramine and citicoline elicited an influence on theoretical %50 % OAT and theoretical %50 TST (theoretical ED50) consistent with an additive interaction. One sample t-test exhibited no significant difference between experimental ED50 and theoretical ED50. Our data showed an additive influence of imipramine and citicoline co-treatment upon induction of anxiolytic-like [t (23) = 0.667, P = 0.511; Fig. 5A] and antidepressant-like [t (23) =0.836, P = 0.429; Fig. 5B] responses in nerve-ligated mice.

4. Discussion

Animal models based on peripheral nerve ligation are used for the assessment of behavioral abnormalities induced by neuropathic (Suzuki et al., 2007). Our study indicated that sciatic nerve ligation induced anxious- and depressant-like behaviors in male mice. According to our study, Suzuki and co-workers (2007) reported that the experimental neuropathy model in mice is correlated with delayed alterations linked to anxiety and depression behaviors (Suzuki et al., 2007).

On the other hand, our research showed that moderate (50 mg/kg) and high (100 mg/kg) doses of citicoline can reverse anxious- and depressant-like effects following sciatic nerve ligation in male mice. Consistent with our results, Abdolmaleki et al. (2016) showed the anxiolytic activity of citicoline in rats (Arcadi et al., 2021). Carlezon

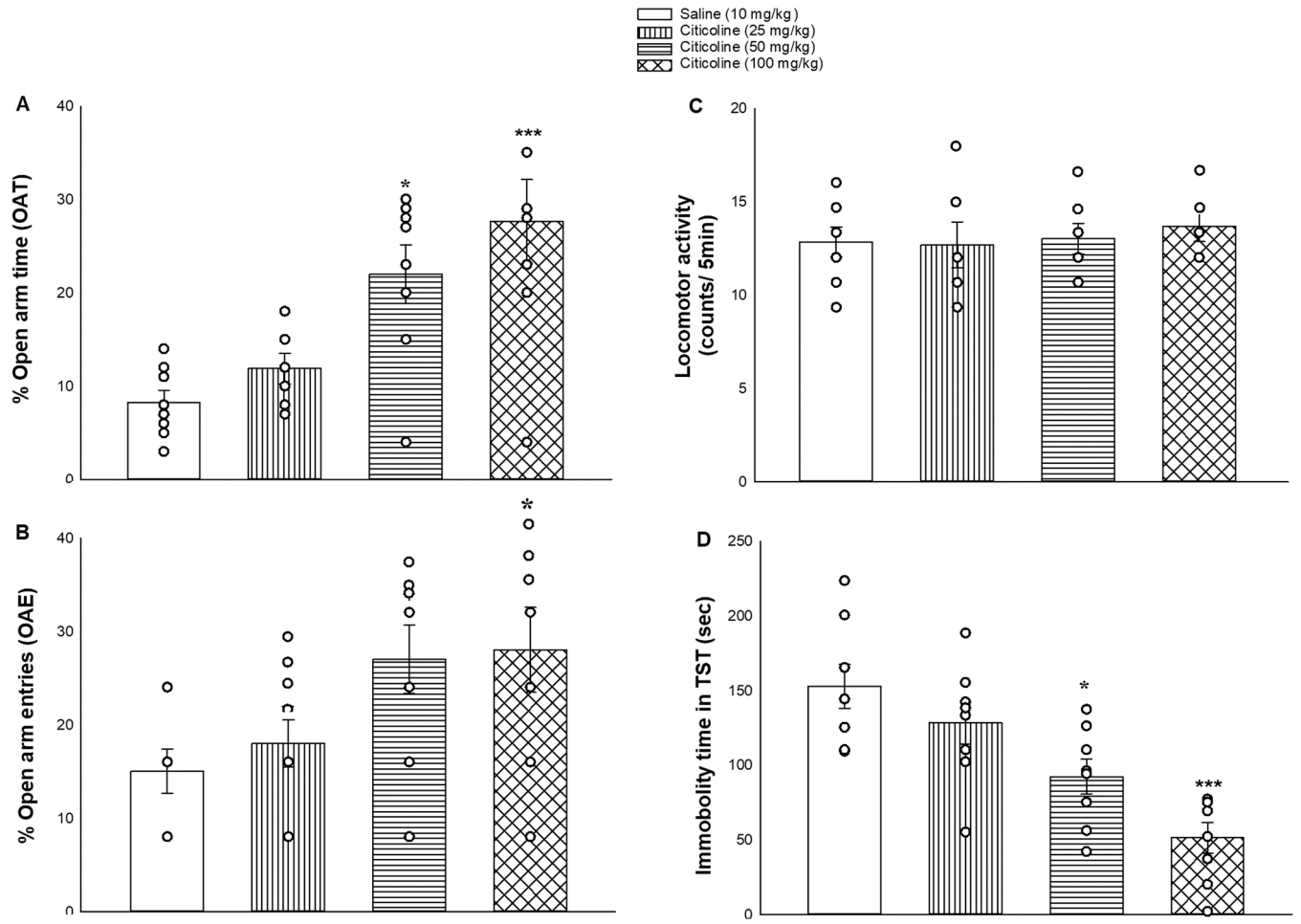

Fig. 3. The effects of different dosages of citicoline at low (25 mg/kg), moderate (50 mg/kg), and high (100 mg/kg) doses on %OAT (A), %OAE (B), locomotor activity (c) in the EPM as well as immobility time of the TST (D) in nerve-ligated mice. Data indicated as mean ± S.E.M. (n = 8). *P < 0.05, ** P < 0.01, and *** P < 0.001 compared to saline group.

et al. (2002) displayed the antidepressant-like response of citicoline in the forced swim test in rats. Also, Roohi-Azizi and coworkers (2018) indicated that citicoline induced antidepressant behavior in male mice (Roohi-Azizi et al., 2018). Citicoline has neuroprotective properties due to the greater accessibility of phosphatidylcholine might activate the repair and regeneration of injured cell membranes in the neurons (Jasielski et al., 2020; Roohi-Azizi et al., 2018). Additionally, once choline is decreased, phospholipids are hydrolyzed to reverse the choline amount. Synthesis of acetylcholine is preferred once the accessible quantity of choline is restricted. Hence, citicoline is a resource of choline, evading phosphatidylcholine hydrolysis (Adibhatla & Hatcher, 2002). Animal and human studies demonstrated the beneficial effect of citicoline in the regeneration of neurons and induces an antidepressant-like response because of the capability to enhancement several neurotransmitter levels such as acetylcholine, dopamine, serotonin, and norepinephrine (Arcadi et al., 2021; Jasielski et al., 2020; Secades, 2016). Citicoline might be an extra agent for the treatment of depression and mood modulation (Brown & Gabrielson, 2012; Brown et al., 2015; Jasielski et al., 2020; Roohi-Azizi et al., 2017). Moreover, citicoline is related to changes in the brain membrane metabolism, which raises the probability that citicoline might have utility as an antidepressant treatment (Carlezon et al., 2002).

Furthermore, the obtained data exhibited that imipramine induced anxiolytic- and antidepressant-like effects in the nerve-ligated mice. According to our results, Paolo and Galistu (2019) exhibited the antidepressant-like behavior of imipramine in the forced swim test of the rat model (Paolo & Galistu, 2019). Yanagida et al. (2016) showed that imipramine elicited an antidepressant-like influence in the forced swim test of mice models. The antidepressant mechanism of imipramine can be associated with its effect on the central monoaminergic pathways (Zarrindast & Sahebgharani, 2002). Additionally, Ramirez and Sheridan (2016) revealed the anxiolytic- and antidepressant-like responses induced by imipramine in mice (Ramirez & Sheridan, 2016). This evidence supports the idea that pharmaco-modulation of the monoaminergic system, also inducing anxiolytic- and antidepressant-like properties, may have therapeutic properties.

Tricyclic antidepressants are administrated either alone or in combination with other compounds to treat mood disorders, thus we decided to assess a probable interaction between imipramine and citicoline on the control of anxiety- and depression-like behaviors in nerve-ligated mice. Our data showed that citicoline can potentiate imipramine anxiolytic- and antidepressant-like effects due to the enhancement of %OAT and %OAE in the EPM as well as reduction of immobility time in the TST in nerve-ligated mice. Interestingly, our results showed an additive influence among imipramine and citicoline on the induction of anxiolytic- and antidepressant-like behaviors in nerve-ligated mice. In this context, our prior investigation exhibited that co-administration of imipramine and citicoline induced an antidepressant-like response in intact male mice (Khakpai et al., 2021).

In this study, to investigate the interaction between citicoline and imipramine, a low dosage of citicoline was used because a high dose of citicoline can affect the response induced by imipramine. But it was

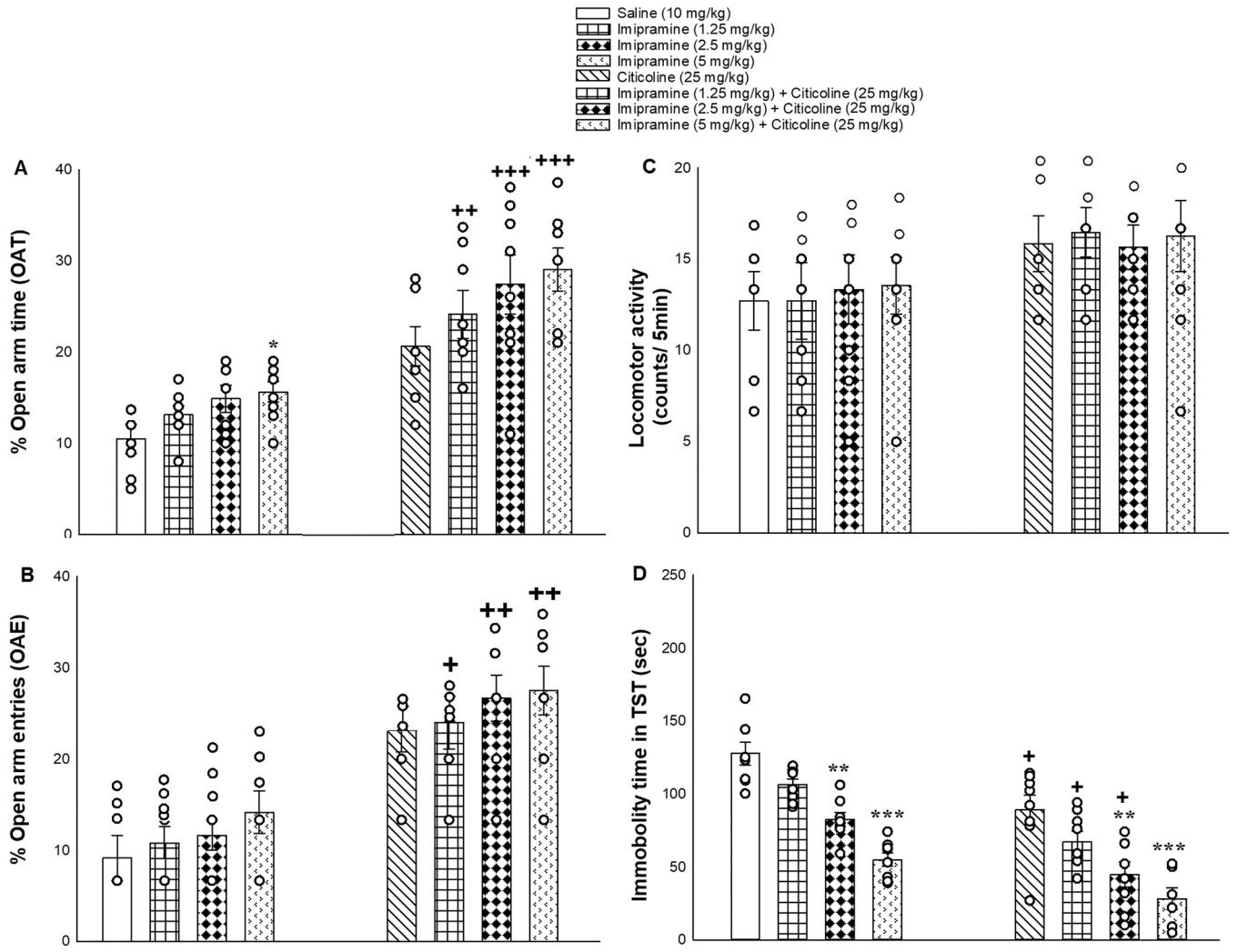

Fig. 4. The effects of alone administration of imipramine at low (1.25 mg/kg), moderate (2.5 mg/kg), and high (5 mg/kg) doses and co-administration of these dosages plus a low dose of citicoline (25 mg/kg) on %OAT (A), %OAE (B), locomotor activity (c) in the EPM as well as immobility time of the TST (D) in nerve-ligated mice. For a better comparison of the effect of a drug with different doses, each drug was shown with the same pattern so that the difference between the low, moderate and high doses of a drug can be easily compared with control group. Data indicated as mean ± S.E.M. (n = 8). *P < 0.05, **P < 0.01, and ***P < 0.001 compared with saline group. + P < 0.05, ++ P < 0.01, and +++ P < 0.001 in comparison to the saline/imipramine at specific dosage group.

possible that a low dose of citicoline could not affect the response induced by imipramine. The results of this study using a low dose of citicoline along with different doses of imipramine showed that a low dose of citicoline was able to affect the response induced by different doses of imipramine, which indicates an interaction effect between the two. In this study, imipramine and citicoline possessed good effectiveness in inducing anxiolytic- and antidepressant-like behaviors. Therefore, the use of new drugs that can increase the effectiveness of anxiolytic and antidepressant effects or decrease their side effects will aid in developing therapeutic approaches for anxiety and depression behaviors. Citicoline elicited effects alike to those induced by tricyclic antidepressants including enhancement of norepinephrine and dopamine levels in the brain (Carlezon et al., 2002). Anxiety and depression treatment may be usually performed via the use of a co-treatment of some substances with different mechanisms of action as a replacement for the application of a single drug (Roohi-Azizi et al., 2018). Consequently, the use of novel drugs and compounds can improve the effectiveness of anxiolytic and antidepressant agents which leads to a decrease in the used dosages and a discount in side effects of long-term administration. Results of this research revealed that citicoline was a real adjuvant to imipramine in the management of anxiety- and depression-like effects in nerve-ligated mice.

Author agreement

M.R. Zarrindast designed the study. B. Hajikarimloo, N. Raissi-Dehkordi, and N. Raissi-Dehkordi acquired the animal data. F. Khakpai was responsible for the study concept and assisted with the interpretation of findings. The authors critically reviewed the content and approved the final version for publication.

CRediT authorship contribution statement

Mohammad-Reza Zarrindast: Resources, Project administration, Methodology. **Bardia Hajikarimloo:** Data curation. **Nastaran Raissi-Dehkordi:** Data curation. **Negar Raissi-Dehkordi:** Data curation. **Fatemeh Khakpai:** Writing – review & editing, Writing – original draft, Validation, Supervision, Software, Resources, Methodology, Investigation.

Compliance with ethical standards

The study was performed under ethical standards in all aspects.

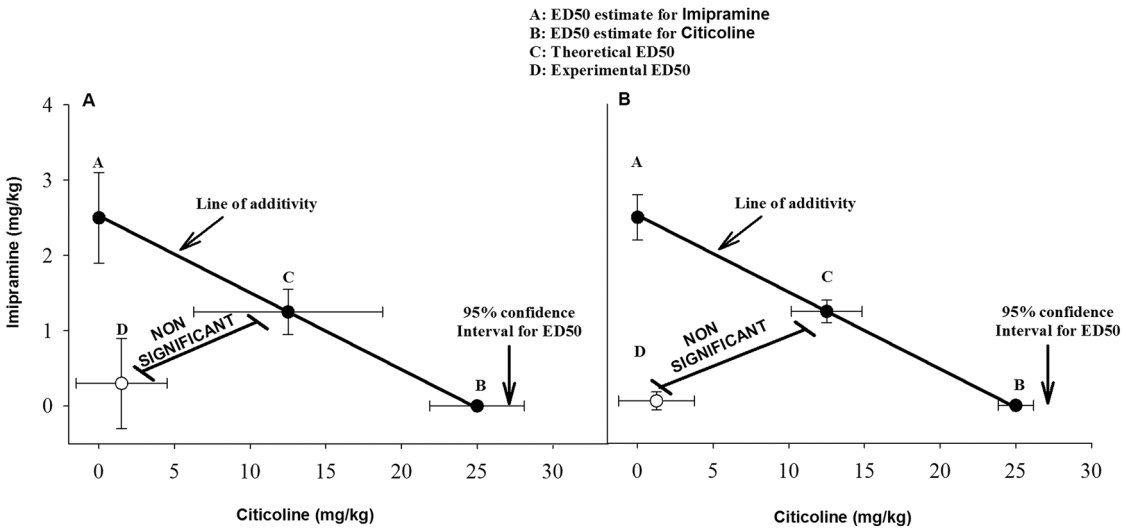

Fig. 5. The isobologram analysis of the effects of drug treatment showed the additive effect of imipramine and citicoline on the induction of anxiolytic and antidepressant-like responses in nerve-ligated mice. Statistical analysis displayed that there is no significant difference between experimental ED50 and theoretical ED50 points, showing an additive effect of the co-treatment of the drugs ((A) for %OAT and (B) for TST. ED50, effective dose 50.

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Conflict of interest

No financial or other conflicts of interest are declared.

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Data availability

All authors declared that all data of this study are available upon request.

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