RESEARCH PAPER

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Pharmacological modulation of anxiety-like and depression-like behaviors in nerve-ligated mice: regarding the effects of citicoline and bupropion

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Neuropathic pain is a condition that results from nerve injury. There is a relationship between neuropathic pain and mood disorders. Citicoline, when used as a dietary supplement, exhibits neuroprotective, antidepressant, and anxiolytic properties. Moreover, bupropion is an atypical antidepressant with unique pharmacologic properties. We sought to investigate the effects of citicoline and bupropion as well as their possible interaction on the control of anxiety- and depression-like behaviors in nerve-ligated mice. Unilateral sciatic nerve ligation was performed on the right hind limb. Anxiety- and depression-like behaviors were measured using the elevated plus-maze and the tail suspension test, respectively. The results showed that sciatic nerve ligation decreased the percentage of time spent in the open arms of the elevated plus-maze and increased the immobility time in the tail suspension test, displaying anxiogenic- and depressant-like responses in the nerve-ligated mice. Intraperitoneal administration of citicoline increased the percentage of time spent in the open arms and diminished the immobility time of the tail suspension test compared to the saline group, showing anxiolytic and antidepressant-like responses. Additionally, the injection of bupropion induced antianxiety- and antidepressant-like responses increasing the percentage of time spent in the open arms and reducing immobility time in nerve-ligated mice. Co-injection of bupropion and citicoline potentiated the antidepressant property of bupropion in nerve-ligated mice. Additionally, we determined an additive effect between bupropion and citicoline on the generation of anxiolytic and antidepressant-like behaviors in the nerve-ligated mice. Based on these results, we concluded that there is a crosstalk between bupropion and citicoline in the control of anxiety- and depression-like behaviors in the nerve-ligated mice.

Key words: bupropion, citicoline, anxiolytic-like behavior, antidepressant-like behavior, nerve-ligated, mice

INTRODUCTION

Cytidine-5-phosphocholine (CDP-choline; citicoline) is similar to the natural intracellular precursor of phospholipid phosphatidylcholine. It is a mononucleotide comprising choline, cytosine, pyrophosphate, and ribose whose chemical structure corresponds to 2-oxy-4-aminopyrimidine (Secades & Gareri, 2022). Citicoline participates in the synthesis of the cell membranes. It is widely available as a dietary supplement. Study in the use of citicoline is reported in neurology and psychiatry (Iulia et al., 2017; Roohi-Azizi et al., 2017). It may elicit antidepressant (Carlezon et al., 2002; Brown & Gabrielson, 2012) and anxiolytic properties (Abdolmaleki et al., 2016). In the animal model of nerve injury and neuropathy, citicoline activated re-

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generation of damaged cell membranes of neurons and reduced pain (Iulia et al., 2017; Roohi-Azizi et al., 2017). Citicoline raises the phospholipid levels within the membranes, and the synthesis of structural phospholipids (Adibhatla & Hatcher, 2005; Brown et al., 2015; Secades, 2016), as well as the concentration of acetylcholine, norepinephrine, dopamine, and serotonin concentration in the brain (Brown & Gabrielson, 2012; Brown et al., 2015). Citicoline has neuroprotective properties (Jasielski et al., 2020). The most frequently presented explanation of the neuroprotective properties of citicoline on the brain is based on the hypothesis that it is a pro-drug that, following injection or ingestion, is sequentially hydrolyzed and dephosphorylated to cytidine (or uridine in humans) and choline. Then, these two metabolites independently enter the brain tissues and are utilized to resynthesize of CDP-choline, which induces neuroprotection effects by supporting the biosynthesis of cellular phospholipids (Grieb, 2014).

Bupropion is a norepinephrine and dopamine reuptake inhibitor without serotonergic action. It is clinically used as an antidepressant (Fava et al., 2005; Shen et al., 2019). Furthermore, clinical studies revealed that bupropion alleviates the neuropathic pain (Semenchuk et al., 2001). Moreover, bupropion has a non-competitive nicotinic acetylcholine receptor antagonist effect (Arias, 2009). Since bupropion does not affect the serotonin and postsynaptic receptors, it is considered an antidepressant with a unique clinical property (Stahl et al., 2004).

Sciatic nerve injury is a relevant procedure for the assessment of nociceptive and emotional reactions of continued neuropathic pain in rodents (Hashemzaei et al., 2017). Studies have indicated an association between neuropathic pain and mood disorders. There is a prevalence of the co-morbidity of these disorders (Dersh et al., 2002). Neuropathic pain is often related to anhedonia (Leitl & Negus, 2016). Anxiety and depression are recognized to control pain perception both in the absence and presence of tissue damage (Hestehave et al., 2020). Research on a longer time course and using tight nerve ligation in rodents showed that long-term neuropathic pain causes an anxiety phenotype (Narita et al., 2006; Suzuki et al., 2007). Moreover, the animal models of neuropathic pain indicated a basic and major plan for the development of effective drugs (Medeiros et al., 2021).

Considering both bupropion and citicoline involvement in the modulation of neurotransmitter levels such as norepinephrine, dopamine, and acetylcholine (Stahl et al., 2004; Arias, 2009; Brown & Gabrielson, 2012; Brown et al., 2015; Leitl & Negus, 2016) as well as inducing neuroprotective properties (Grieb, 2014; Jasielski et al., 2020; Vismara et al., 2022), this research was designed to assess the effect of citicoline and bupropion as well as their possible interaction on the control of anxiety- and depression-like responses in the nerve-ligated mice.

METHODS

Animals

Naval Medical Research Institute (NMRI) male mice (6-8 weeks old and 20–25 g weight) were obtained from the Tehran University of Medical Sciences, where they are being bred. Experiments were conducted on the locally bred (Iranian) strain of laboratory mice. To avoid variations in sexual hormones during the estrus cycle, we used only male mice. Mice were kept in the stainless-steel cages at 22±2°C and a 12 h light/dark cycle (lights on at 7 a.m.). Experiments were performed during the light phase between 8 a.m. and 12 p.m. Mice were kept four per cage. They had free access to food and water. Mice were treated under the guidelines for the care and use of laboratory animals according to the Animal Research Ethics Committee of the Tehran University of Medical Sciences (NIH publications No. 80-23).

Surgical procedures

Mice were anesthetized using ketamine (50 mg/kg; i.p.) / xylazine (4 mg/kg; i.p.). Then, unilateral peripheral mononeuropathy was elicited on the right hind limb, according to our previous research (Zarrindast et al., 2000). The mouse's right sciatic nerve was exposed, and a 2 mm long nerve part was dissected at the right hind limb. Only one ligature of fine wire was utilized near to the right hind limb of the severed nerve. Sham-operated mice received the above procedure without the dissection of the sciatic nerve. Mice were singly housed after the surgery. Each mouse was allowed about 5–7 days to recover from surgery and the effect of the anesthetic drugs. Thus, ketamine did not affect the overall results of the study.

Drug treatment

The drugs used for this research included bupropion hydrochloride (Sigma–Aldrich, St. Louis, MO, USA) and citicoline sodium (Minoo, Tehran, Iran). All drugs were dissolved in a sterile 0.9% NaCl solution. The drugs were administered intraperitoneally (i.p.) at a volume of 10 ml/kg. Control mice received sterile saline

(10 ml/kg). We selected the doses of drugs and route of drug administration according to our previous studies (Khakpai & Zarrindast, 2023).

Behavioral tests

Open field test (OFT)

The open field apparatus (Borj Sanat, Tehran, Iran) was a clear perspex box (50 cm × 50 cm × 50 cm). This device had a gray perspex panel (30 cm × 30 cm × 2 cm thick) with 16 photocells, which divided the device into 16 equal-size squares. The number of crossings with all paws from one square to another and time spent in the middle squares of the apparatus were recorded during 5 min.

Elevated Plus Maze (EPM)

We used the elevated plus maze (EPM) apparatus to assess anxiety-like behavior. This technique has been used widely to study novel anxiolytic compounds. The EPM apparatus had two opposing open (30 cm × 5 cm × 1 cm) and two opposing closed arms (30 cm × 5 cm × 15 cm) joined by a common central part (5 cm × 5 cm). This apparatus was elevated 40 cm above the floor. Open and closed arms, as well as the central part, were exposed to about the same brightness (40 lux, with no shades). Arm entry or exit were scored when all paws were in or out of an arm, respectively. Findings were considered as follows: %OAT (ratio of time spent in the open arms to the total time spent in the open and closed arms × 100); %OAE (ratio of entries into the open arms to the total entries into the open and closed arms × 100). The number of total arm entries was considered as locomotor activity. Each mouse was gently placed on the central platform of the EPM apparatus. For 5 min, %OAT, %OAE, and locomotor activity were measured (de Figueiredo Cerqueira et al., 2023).

Tail suspension test (TST)

We also used the tail suspension test (TST) to measure depression-like behavior. In the TST, the whole time of immobility elicited *via* tail suspension was recorded. The mouse was suspended 50 cm above the floor utilizing adhesive tape located approximately 1 cm from the tip of the mouse's tail. In this test, mice allowed 2 min for habituation, and in the remaining 4 min, immobility time was measured (Haj-Mirzaian et al., 2015). The mouse was determined to be immobile when it did not display any movement of the body for 4 min (Dhingra & Sharma, 2006).

Experimental design

To habituate mice to the test room, they were moved into the experimental room 30 min before the test session. About 10 min after drug administration, behavioral tests were performed. Locomotor activity and anxiety tests were performed for 5 min, and the depression test was performed for 6 min. A blinded of test experimenter carefully recorded all behavioral tests using video. Eight mice were used for each experimental group. In groups that mice received injections of two drugs, the drugs were injected separately. The control group received two saline injections. This investigation comprised of four experiments.

In experiment 1, the effect of sciatic nerve ligation on the %OAT, %OAE, and locomotor activity in the EPM and the immobility time of the TST were examined.

In experiment 2, the influence of saline injection (10 ml/kg; i.p.) and diverse doses of citicoline (25, 50, 75, and 100 mg/kg; i.p.) on the %OAT, %OAE, and locomotor activity in the EPM and the immobility time of the TST were examined.

In experiment 3, the influence of injection of saline only (10 ml/kg; i.p.) or of diverse doses of bupropion (1.25, 2.5, and 5 mg/kg; i.p.), as well as co-injection of diverse doses of bupropion (1.25, 2.5, and 5 mg/ kg; i.p.) with an ineffective dose of citicoline (25 mg/kg; i.p.), were examined on the performance of mice in the EPM and TST.

Experiment 4, the effects of co-injection of bupropion 2.5 mg/kg + citicoline 50 mg/kg, bupropion 1.25 mg/kg + citicoline 25 mg/kg, and bupropion 0.625 mg/kg + citicoline 12.5 mg/kg on anxiety- and depression-related behaviors were tested. Table 1 explained the experimental groups.

Statistical analysis

Statistical analyses were performed using the SPSS statistical package. Results were presented as the mean ± standard error of the mean (SEM). Data from the experiments 1 to 3 were evaluated using one-way and two-way analysis of variance (ANOVA) followed by the Tukey *post hoc* test to determine differences among the treatments. $P<0.05$ showed statistically significant.

Additionally, an isobolographic test (experiment 4) was performed to detect the interaction after the administration of two substances. The parallelism between dosage-response curves of a single drug (bupropion or citicoline) and those of the same drug in the presence of a fixed quantity of another one (citicoline or bupropion) was examined. There are other

Table 1. 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), bupropion (1.25, 2.5, and 5 mg/kg)	Anxiolytic	–
	A (Right panel)	Saline (10 ml/kg), bupropion (1.25, 2.5, and 5 mg/kg) + citicoline (25 mg/kg)	Anxiolytic	–
	B (Left panel)	Saline (10 ml/kg), bupropion (1.25, 2.5, and 5 mg/kg)	–	–
	B (Right panel)	Saline (10 ml/kg), bupropion (1.25, 2.5, and 5 mg/kg) + citicoline (25 mg/kg)	Anxiolytic	–
	C (Left panel)	Saline (10 ml/kg), bupropion (1.25, 2.5, and 5 mg/kg)	–	–
	C (Right panel)	Saline (10 ml/kg), bupropion (1.25, 2.5, and 5 mg/kg) + citicoline (25 mg/kg)	–	–
	D (Left panel)	Saline (10 ml/kg), bupropion (1.25, 2.5, and 5 mg/kg)	–	Antidepressant
	D (Right panel)	Saline (10 ml/kg), bupropion (1.25, 2.5, and 5 mg/kg) + citicoline (25 mg/kg)	–	Antidepressant
4	A	Bupropion 2.5 mg/kg + citicoline 50 mg/kg Bupropion 1.25 mg/kg + citicoline 25 mg/kg Bupropion 0.625 mg/kg + citicoline 12.5 mg/kg	Additive anxiolytic	–
	B	Bupropion 2.5 mg/kg + citicoline 50 mg/kg Bupropion 1.25 mg/kg + citicoline 25 mg/kg Bupropion 0.625 mg/kg + citicoline 12.5 mg/kg	–	Additive antidepressant

methods wherein single-drug dosage-response relations are compared with dosage-response relations for which the dosages of both drugs vary, but their ratio is preserved fixed. For single-drug dosage-response curves of same slope and administration a fixed dosage ratio of the two drugs combined, both additive and non-additive combination relations are parallel to the single-drug relations. This means that deviation of additivity is not showed *via* a deviation from parallelism but *via* the quantity of the shift in comparison to the parallel additivity line (Sühnel, 1998). Moreover, the interaction index amounts for the examined two-drug combinations were determined as a ratio of the experimental ED50 to the theoretical ED50 amounts following first drug (ED50/2 mg/kg) + second drug (ED50/2 mg/kg); first drug (ED50/4 mg/kg) + second drug (ED50/4 mg/kg) and first drug (ED50/8 mg/kg) + second drug (ED50/8 mg/kg).

The ED50 of each drug (2.5 mg/kg for bupropion and 50 mg/kg for citicoline) was assessed with a linear regression test. A mixture of two drugs was injected in a fixed dose ratio according to the ED50 amounts. For drug co-treatment, the theoretical ED50 is bupropion ED50/2 + citicoline ED50/2. Furthermore, experimental data of drug co-treatment from constant ratio-measured were tested using a regression test, after which the experimental ED50 data of the drug co-treatment were assessed (%50 %OAT of the EPM and % 50% immobility time of the TST). A one-sample t-test was performed to assess the statistical significance of the difference between the theoretical ED50 and experimental ED50 of drug co-treatment. If experimental ED50 was significant lower than theoretical ED50, a synergistic interaction between bupropion and citicoline might have been considered; however, there was no difference among them, showing an additive interaction rather than a synergistic response. Alterations with $P<0.05$ among experimental groups at each point were detected as statistically significant.

RESULTS

The effect of sciatic nerve ligation on the anxiety- and depression-like behaviors

In Fig. 1, the influence of sciatic nerve ligation on the anxiety- and depression-like responses in male mice is shown. One-way ANOVA and *post hoc* test indicated that sciatic nerve ligation decreased the %OAT [$F_{(2,21)}=4.966$, $p=0.017$; Fig. 1A] and %OAE [$F_{(2,21)}=4.710$, $p=0.021$; Fig. 1B] but increased immobility time of the TST [$F_{(2,21)}=4.764$, $P=0.020$; Fig. 1E] compared to the control and sham groups. No change in locomotor activity number was observed [$F_{(2,21)}=0.175$, $p=0.841$; Fig. 1C]. However, there is a significant difference between groups for time spent in the middle squares [one-way ANOVA followed by *post hoc* test: $F_{(2,21)}=3.948$, $p=0.035$; Fig. 1D], suggesting anxiety-like behavior in the sciatic nerve ligated mice.

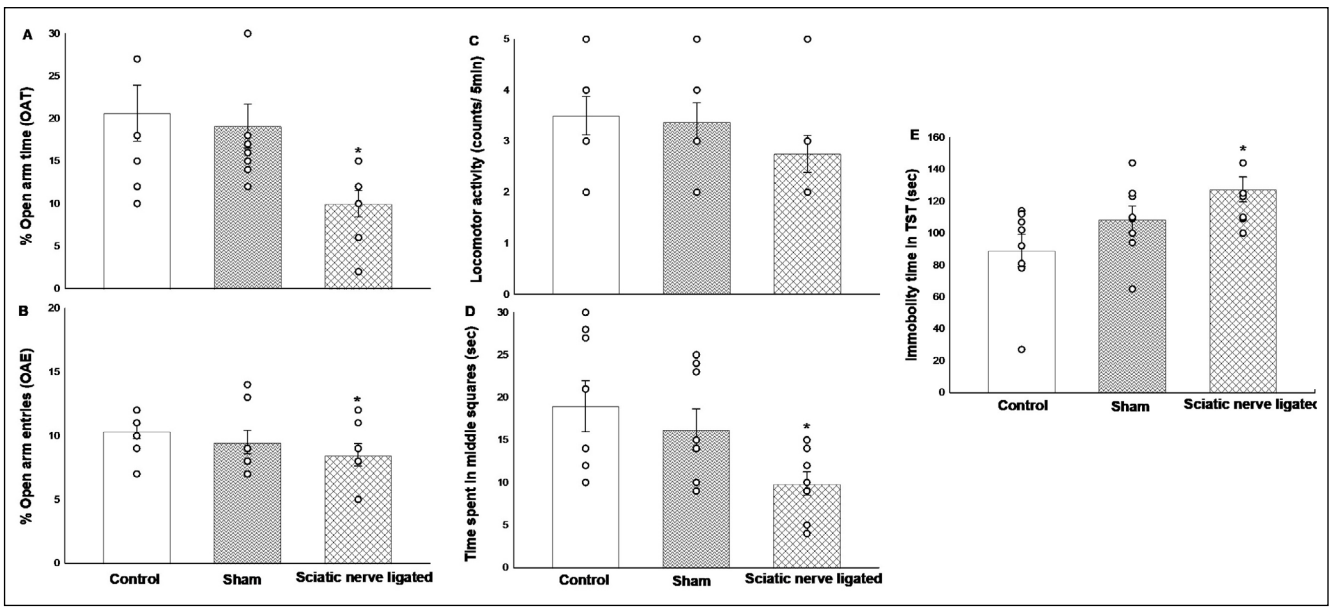

Fig. 1. The effect of sciatic nerve ligation on anxiety- and depression-like behaviors on %OAT (A), %OAE (B), locomotor activity (C) in the EPM, as well as immobility time of the TST (D) in nerve-ligated mice. Data presented as mean ± S.E.M. (n=8). *P<0.05 compared with control and sham groups.

Effect of citicoline on the anxiety- and depression-like behaviors

Fig. 2 indicated the influence of different doses of citicoline (25, 50, 75, and 100 mg/kg) on the anxiety- and depression-like behaviors using the EPM and TST in the nerve-ligated mice. One-way ANOVA and *post hoc* test showed that i.p. treatment with citicoline raised the %OAT [$F_{(4,35)}$=10.453, p=0.000; Fig. 2A] but decreased immobility time of the TST [$F_{(4,35)}$=7.329, P =0.000; Fig. 2E] at the doses of 75 and 100 mg/kg compared to the saline group. These dosages of citicoline had no significant influence on %OAE [$F_{(4,35)}$=2.403, p=0.068; Fig. 2B] and locomotor activity [$F_{(4,35)}$=0.265, p=0.905; Fig. 2C]. Nevertheless, there is a significant difference between groups for time spent in the middle squares [one-way ANOVA followed by *post hoc* test: $F_{(4,35)}$=3.007, p=0.031; Fig. 2D], proposing antianxiety-like effect of citicoline at the dose of 100 mg/kg compared to the saline group.

Effect of bupropion alone or with citicoline on the anxiety- and depression-like behaviors

The effects of different doses of bupropion (1.25, 2.5, and 5 mg/kg) and co-injection of diverse doses of the drug, plus a sub-threshold dosage of citicoline (25 mg/kg) on the anxiety- and depression-like responses in the nerve-ligated mice are shown in Fig. 3. One-way ANOVA and *post hoc* test showed that bupropion at the dose of 5 mg/kg enhanced the %OAT [$F_{(3,28)}$=4.055, p=0.016; Fig. 3A, left panel] and the time spent in the middle squares [$F_{(3,28)}$=4.751, p=0.008; Fig. 3D, left panel] in comparison to the saline group. Also, bupropion at the doses of 2.5 and 5 mg/kg decreased immobility time of the TST [$F_{(3,28)}$=12.255, P =0.000; Fig. 3E, left panel] compared to the saline group. These treatments had no significant influence on the %OAE [$F_{(3,28)}$=2.546, p=0.084; Fig. 3B, left panel] and locomotor activity [$F_{(3,28)}$=0.408, p=0.748; Fig. 3C, left panel].

Two-way ANOVA showed no significant interaction among bupropion and citicoline on the %OAT [treatment effect: $F_{(1,56)}$=46.292, P=0.000, dose effect: $F_{(3,56)}$=7.054, P=0.000, treatment–dose interaction: $F_{(3,56)}$=0.562, P=0.642; Fig. 3A; right panel], %OAE [treatment effect: $F_{(1,56)}$=48.645, P=0.000, dose effect: $F_{(3,56)}$=4.015, P=0.012, treatment–dose interaction: $F_{(3,56)}$=0.225, P=0.879; Fig. 3B; right panel], locomotor activity of the EPM [treatment effect: $F_{(1,56)}$=0.768, P=0.385, dose effect: $F_{(3,56)}$=0.064, P=0.979, treatment–dose interaction: $F_{(3,56)}$=0.448, P=0.719; Fig. 3C; right panel], the time spent in the middle squares [treatment effect: $F_{(1,56)}$=45.426, P=0.000, dose effect: $F_{(3,56)}$=13.759, P=0.000, treatment–dose interaction: $F_{(3,56)}$=0.374, P=0.772; Fig. 3D; right panel], and immobility time of TST [treatment effect: $F_{(1,56)}$=63.752, P=0.000, dose effect: $F_{(3,56)}$=38.147, P=0.000, treatment–dose interaction: $F_{(3,56)}$=2.267, P=0.091; Fig. 3E; right panel]. On the other hand, *post hoc* test showed that co-treatment of with bupropion (2.5 mg/kg) and citicoline (25 mg/kg) significantly raised the %OAT and %OAE in the EPM and the time spent in the middle squares of the OFT but re-

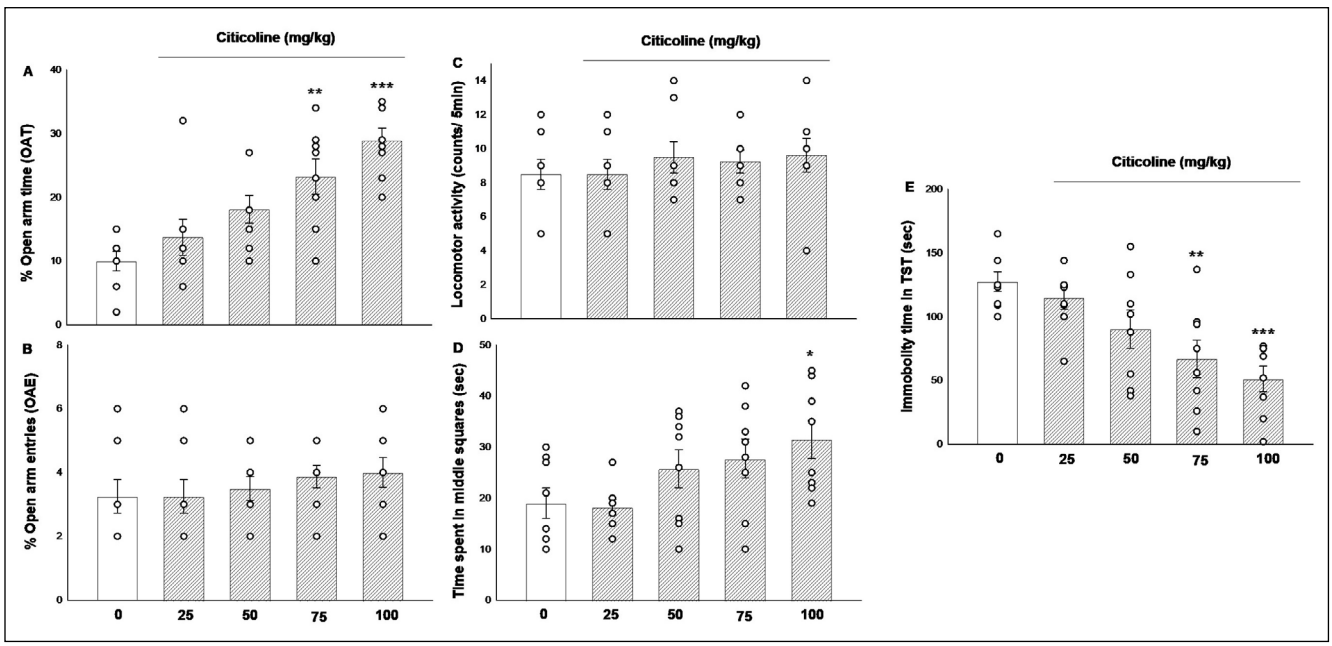

Fig. 2. The effects of saline (10 ml/kg; i.p.) and diverse doses of citicoline (25, 50, 75, and 100 mg/kg) on %OAT (A), %OAE (B), locomotor activity (C) in the EPM, as well as immobility time of the TST (D) in nerve-ligated mice. Data presented as mean ± S.E.M. (n=8). **P<0.01 and ***P<0.001 compared to saline group.

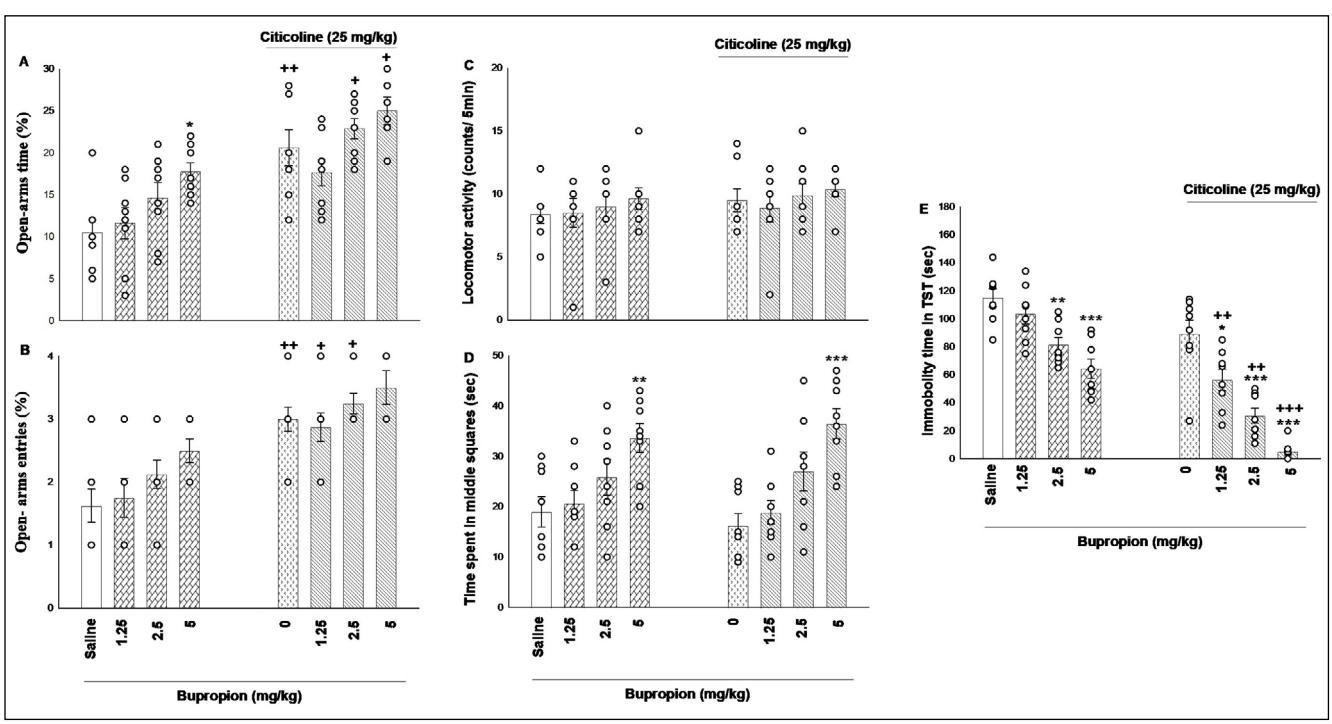

Fig. 3. The effects of saline (10 ml/kg; i.p.), the administration of bupropion (1.25, 2.5, and 5 mg/kg), and co-administration of these doses along with citicoline (25 mg/kg) on %OAT (A), %OAE (B), locomotor activity (C) in the EPM, as well as immobility time of the TST (D) in nerve-ligated mice. Data presented 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/bupropion group.

duced immobility time in the TST. These results showed that injection of an ineffective by itself dose of citicoline along with bupropion could strengthen the effect of bupropion in the enhancement of %OAT, %OAE, and the time spent in the middle squares, showing antianxiety-like effect.

The additive effect between bupropion and citicoline on anxiolytic- and antidepressant-like responses

Theoretical additive line exhibited at all points, that bupropion and citicoline co-treatment influenced the theoretical %50 %OAT and theoretical %50 of the TST (theoretical ED50) based on an additive interaction. One-sample t-test showed no significant difference between experimental ED50 and theoretical ED50. The results indicated an additive influence of bupropion and citicoline co-treatment on inducing anxiolytic-like [t(23)=0.949, P=0.353; Fig. 4A] and antidepressant-like [t(23)=0.771, P=0.449; Fig. 4B] responses in the nerve-ligated mice.

DISCUSSION

The results of this study showed that sciatic nerve ligation decreased the %OAT but increased the immobility time of the TST, showing anxiogenic- and depressant-like effects in the nerve-ligated mice. Also, the locomotor activity of nerve-ligated mice was changed, but this change was not significant. Consistent with other studies, our results indicated that ligation of the sciatic nerve elicited anxiety- and depression-like phenotype in the nerve-ligated mice (Leitl & Negus, 2016; Narita et al., 2006; Suzuki et al., 2007).

It is demonstrated that females show different pain sensitivity in physiological and pathological situations in comparison to males (Zang et al., 2020). There is evidence that gender, gonadectomy, as well as estrus phases of female rodents affect pain sensitivity, anxiety and depression behaviors (Frye et al., 1993; Behr et al., 2009; Ibironke & Aji, 2011). Also, there are sex-related differences in the antinociceptive effects of opioids which indicated the importance of rat genotype, nociceptive stimulus intensity, and efficacy at the μ opioid receptor (Cook et al., 2000; Barrett, 2006). Furthermore in humans, females seek pain relief and suffer sciatic pain more frequently than males (Peul et al., 2008). The behavior of female rodents in tasks assessing pain sensitivity changes by the estrus cycle phases (Frye et al., 1993). Hence, male mice were only used in this research to avoid the impacts of variable levels of sexual hormones on the pain sensitivity in the female sexual cycle (Stoffel et al., 2003).

The results of this investigation showed that i.p. treatment with citicoline showed a significant enhancement in %OAT but not %OAE and locomotor activity, in accordance with an anxiolytic-like influence. Additionally, i.p. treatment with citicoline dose-dependently diminished immobility time in the TST which showed anxiolytic- and antidepressant-like effects. Citicoline is a neuroprotective substance that is used after ischemic and traumatic brain damage. There is little evidence of anxiolytic and anti-depressive effect of citicoline, which are addressed in this investigation. Following administration, citicoline

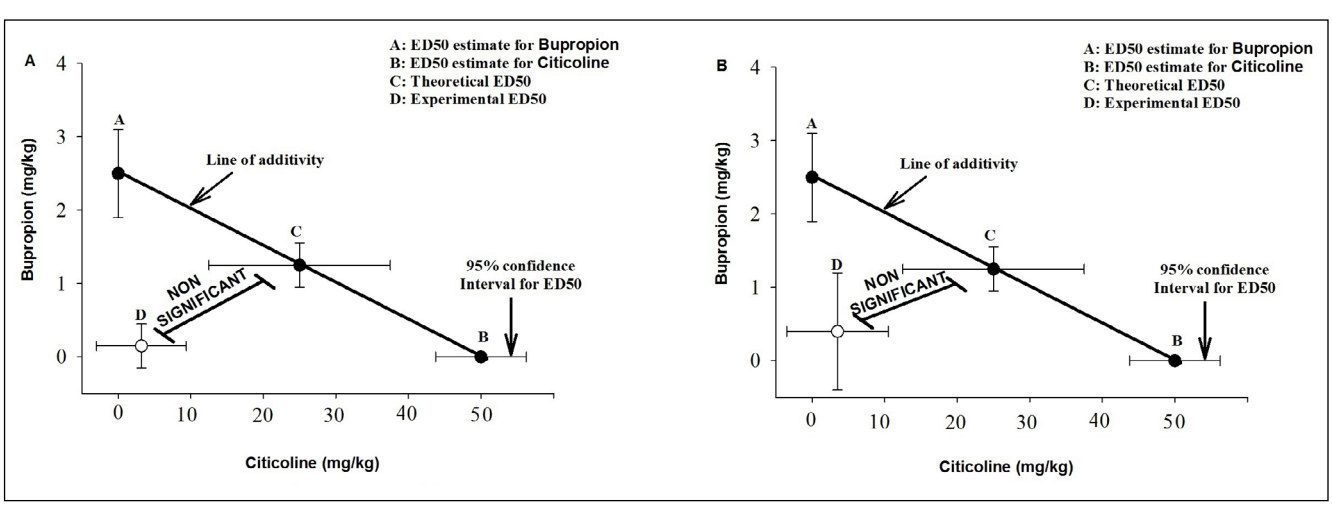

Fig. 4. The isobologram analysis of the effects of drug treatment revealed the additive effect of bupropion and citicoline on the induction of anxiolytic and antidepressant-like effects in nerve-ligated mice. Statistical analysis indicated that there is no significant difference between experimental ED50 and theoretical ED50 points, presenting an additive effect of the co-injection of the drugs (A) for %OAT and (B) for TST. ED50, effective dose 50.

rapidly breaks into cytidine and choline, that crosses from the blood-brain barrier independently and that reconvert to citicoline within the nerve cells (Galletti et al., 1991). Then, citicoline stabilizes the cell membranes through activation of the synthesis of structural phospholipids, mainly phosphatidylcholine. Phosphatidylcholine is an important factor for the cell membrane integrity and restoration (Zweifler, 2002; Arenth et al., 2011; Wignall & Brown, 2014). Other neuroprotective mechanisms of citicoline include the reduction of generation of the free radicals and reinforcement of the intracellular anti-oxidative system (Adibhatla et al., 2001). Additionally, citicoline influences neurotransmitter amounts mostly through the control of catecholaminergic neurotransmission (Agut et al., 2000). It acts as a dopaminergic agent. What more, citicoline has some influence on the other monoamines, norepinephrine, and serotonin concentrations, as well as cholinergic, glutamatergic, and GABAergic receptors (Dowd et al., 2001). It inhibits the catabolism of the brain phospholipids and induces a protective influence on the membrane ATPase and other enzymes involved in the brain metabolism, principally succinyl dehydrogenase and citrate synthetase (Secades & Lorenzo, 2006). Our results indicate that anxiolytic- and antidepressant-like effects elicited by citicoline depend on its neuroprotective property and capability to enhance serotonergic and noradrenergic transmission. Our results are in accordance with the results some studies reporting that i.p. administration of citicoline induced antianxiety- and anti-depressive-like effects (Abdolmaleki et al., 2016; Roohi-Azizi et al., 2018).

Moreover, the results showed that bupropion induced anxiolytic- and antidepressant-like responses by enhancement of the %OAT and reduction of the immobility time in the nerve-ligated mice. Microdialysis research that recorded neurotransmitter amounts in the nucleus accumbens of mice indicated that extracellular dopamine and norepinephrine concentrations were enhanced in reaction to the bupropion treatment (Nomikos et al., 1989; 1992). On the other hand, the administration of dopamine- or norepinephrine-blocking agents decreased the antidepressant property of bupropion (Cooper et al., 1980). Moreover, animal investigation indicated that bupropion raises the monoaminergic neurotransmission differently from other antidepressants (Ferris & Cooper, 1993). For example, in animals (specially rodents) studies, bupropion did not change the serotonergic transmission either presynaptically (*via* influencing serotonin release and reuptake) or post-synaptically (*via* coupling to the serotonin receptors) (Ferris & Cooper, 1993; Stahl et al., 2004). Studies reported that the acute treatment with bupropion dose-dependently decreased the activity of the dopaminergic and norepinephrinergic neurons in the brain stem of rodents (Ferris & Cooper, 1993; Ascher et al., 1995), a result consistent with the rise in the synaptic amount of dopamine and norepinephrine, which prevents the neuronal activity through an autoreceptor-produced negative feedback effect. Theses preclinical investigations revealed that the action mechanism of bupropion most likely includes its dual-reuptake prevention of dopamine and norepinephrine neurotransmitters (Stahl et al., 2004; Leitl & Negus, 2016). Hence, the antianxiety- and anti-depressive-like effects of bupropion are related to its action as the dual-reuptake inhibition of norepinephrine and dopamine neurotransmitters.

Additionally, our results indicated a crosstalk between bupropion and citicoline for inducing of anxiolytic- and antidepressant-like responses in the nerve-ligated mice. Furthermore, we found an additive response between bupropion and citicoline on the inducing of antianxiety- and anti-depressive-like responses in the nerve-ligated mice. As the therapeutic properties of antidepressant drugs are produced *via* modifying the neurotransmitter levels, a cross-talk between bupropion and citicoline might be due to the enhancement of various neurotransmitter levels including norepinephrine, dopamine, and acetylcholine (Stahl et al., 2004; Arias, 2009; Brown & Gabrielson, 2012; Brown et al., 2015; Leitl & Negus, 2016) which caused anxiolytic- and antidepressant-like behaviors in the nerve-ligated mice. Nonetheless, further investigations are required to clarify the precise mechanism of interaction between bupropion and citicoline on the management of anxiety- and depression-like behaviors in the nerve-ligated mice.

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