Pharmacology, Biochemistry and Behavior 180 (2019) 92–100

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

Simvastatin exerts antidepressant-like activity in mouse forced swimming test: Role of NO-cGMP-K_{ATP} channels pathway and PPAR-gamma receptors

Reza Naserzadeh[a], Niloofar Abad[a], Behnam Ghorbanzadeh[a,*], Mojtaba Dolatshahi[b], Mohammad Taghi Mansouri[c]

[a] Department of Pharmacology, School of Medicine, Dezful University of Medical Sciences, Dezful, Iran
[b] Department of Physiology, School of Medicine, Dezful University of Medical Sciences, Dezful, Iran
[c] Department of Anesthesiology, Columbia University Medical Center, New York, NY 10032, USA

ARTICLE INFO

Keywords:
Simvastatin
Anti-depressant-like
NO-cGMP-K_{ATP} channels pathway
PPARγ receptors
Potentiation
Mouse forced swimming test

ABSTRACT

Simvastatin, one of the lipophilic statins, has been shown to be effective in reducing depression in rodents. The present study aimed to investigate the potential antidepressant-like activity of simvastatin and the possible involvement of NO-cGMP-K_{ATP} channels pathway and PPARγ using forced swimming test (FST) in mice. In addition, the interaction between simvastatin and fluoxetine as a reference drug was examined. After assessment of locomotor behavior in the open-field test (OFT), FST was applied for evaluation of depressive behavior in mice. Simvastatin at doses (20, 30, and 40 mg/kg, i.p.) was administrated 30 min before the OFT or FST. To evaluate the involvement of NO-cGMP-K_{ATP} channels pathway, mice were pre-treated intraperitoneally with L-arginine (a nitric oxide precursor, 750 mg/kg), L-NAME (a NOS inhibitor, 10 mg/kg), methylene blue (guanylyl cyclase inhibitor, 20 mg/kg), sildenafil (a PDE-5 inhibitor, 5 mg/kg), glibenclamide (ATP-sensitive K^+ channel blocker, 1 mg/kg), and diazoxide (K^+ channels opener, 10 mg/kg). Moreover, to clarify the probable involvement of PPARγ receptors, pioglitazone, a PPARγ agonist (5 mg/kg, i.p.), and GW9662, a PPARγ antagonist (2 mg/kg, i.p.), were pre-treated with simvastatin. Immobility time was significantly decreased after simvastatin injection. Administration of L-NAME, methylene blue, glibenclamide and pioglitazone in combination with the sub-effective dose of simvastatin (20 mg/kg, i.p.) reduced the immobility time in the FST compared to drugs alone, while co-administration of effective doses of simvastatin (30 mg/kg, i.p.) with L-arginine, sildenafil, diazoxide, and GW9662 prevented the antidepressant-like effect of simvastatin. In addition, simvastatin (20 mg/kg) potentiated the antidepressant-like effect of fluoxetine through the NO pathway. None of the drugs produced any significant alterations in locomotor activity using OFT. These results demonstrated that NO-cGMP-K_{ATP} channels pathway and PPARγ receptors may be involved in the antidepressant-like effect of simvastatin.

1. Introduction

Depression is one of the most common psychological disorders, which is characterized by altered mood and aversion resulting in persistent sadness, irritability, anxiousness, pain, insomnia and decrease in concentration during activities (Renaud and Bédard, 2013). Extensive use of antidepressant drugs usually shows no response at therapeutic levels due to neuronal adaptation. Therefore, the development of new antidepressants with known mechanisms of action is valuable in psychiatric disorders (Kay and Atiq, 2006).

Simvastatin, a lipophilic statin, is normally used for reducing the serum levels of cholesterol. Cholesterol has a role in the structure and function of cell membranes and contributes to mood state and response to antidepressants (Pucadyil and Chattopadhyay, 2006). Although the main source of central nervous system (CNS) cholesterol is de novo synthesis within neuronal cells, the increased transfer of plasma cholesterol to the brain may be related to the onset of neurodegeneration (Dietschy and Turley, 2001). Further, Vevera et al. (2016) reported that simvastatin affected membrane fluidity and different cellular processes which are regulated by cholesterol, such as transmembrane transport of serotonin (SERT). Moreover, statins also have some cholesterol-independent effects (Ludka et al., 2013) as neuroprotection against traumatic brain injury (Lu et al., 2004) and seizures (Piermartiri et al., 2009). Previous reports showed that statins such as atorvastatin and lovastatin induces anti-depressive effect and/or potentiates antidepressant drugs indicating a link between the serotonergic system and

* Corresponding author at: School of Medicine, Dezful University of Medical Sciences, Dezful, Iran.
E-mail addresses: b_ghorbanzadeh82@yahoo.com, ghorbanzadeh.b@dums.ac.ir (B. Ghorbanzadeh).

https://doi.org/10.1016/j.pbb.2019.03.002
Received 25 January 2019; Received in revised form 7 March 2019; Accepted 7 March 2019
Available online 08 March 2019
0091-3057/

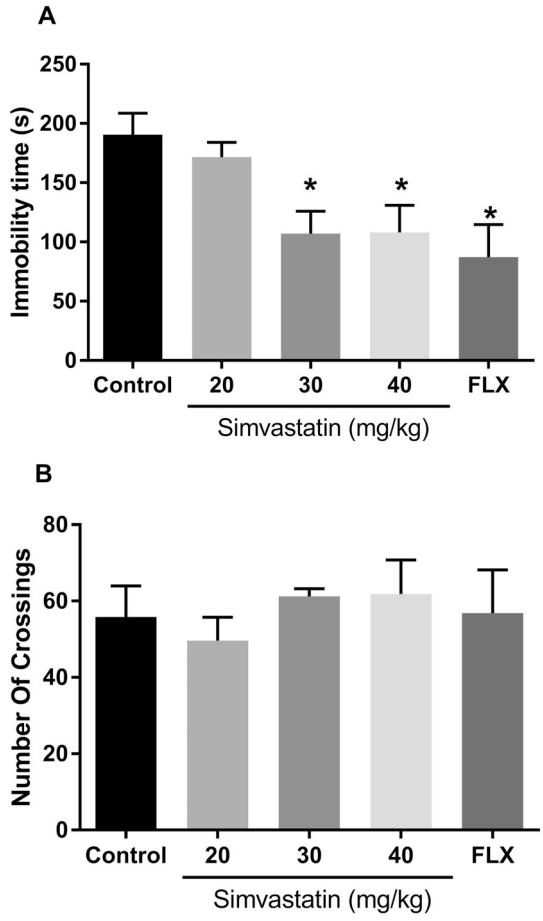

Fig. 1. Effect of administration of simvastatin (20–40 mg/kg, i.p.) in the immobility time in the FST (A) and the number of line crossing in the open-field test (B) in mice. Fluoxetine (FLX) was shown as a positive control drug. Simvastatin or fluoxetine (i.p.) was administered 30 min before the tests. Values are expressed as mean ± S.E.M. (n = 6–10). *P < 0.05 compared with the vehicle-treated control group (one-way ANOVA followed by Tukey's test).

statins (Shahsavarian et al., 2014; Renshaw et al., 2009). Furthermore, simvastatin also produces an antidepressant effect, however, there is little known about its mechanism of action (ElBatsh, 2015; Lin et al., 2014).

A growing body of evidence indicated that nitric oxide (NO)-cGMP-K_{ATP} pathway, an important signaling pathway in CNS such as pain, learning, memory, and depression (Esplugues, 2002; Ghorbanzadeh et al., 2018). NO is produced from L-arginine by the catalytic action of NO synthase. NO actions may be mediated by locally produced NO directly and, also by the subsequently generated guanosine 3′5′ cyclic monophosphate (cGMP). Moreover, studies indicate that different types of K^+ Channels can be activated by NO directly or through cGMP production (Jeong et al., 2001; Yildiz et al., 2000). Also, previous studies demonstrated that the antidepressant-like effect produced by K^+ channels blockade is dependent on the inhibition of NO-cGMP synthesis (Kaster et al., 2005). So, K^+ channels might be one of the targets of NO and the inhibition of these channels may play an important role in depressive disorder (Jeong et al., 2001).

Evidences have indicated that peroxisome proliferator-activated receptor-gamma (PPARγ) is present in different regions of the brain such as hippocampus, basal ganglia, frontal cortex, hypothalamus and pituitary (Cimini et al., 2005; Inestrosa et al., 2005). Previous studies have revealed that PPARγ agonists improve different central nervous system disorders like Alzheimer's disease (Sundararajan et al., 2005), multiple sclerosis and depression (Feinstein et al., 2002; Shahsavarian et al., 2014). Moreover, Rosa et al. (2008) showed that PPARγ receptors are associated with the process of attenuation of depression (Rosa et al., 2008). In our previous study, we observed simvastatin produced antinociception that partly mediated through the activation of PPARγ receptors (Mansouri et al., 2017). Further, it has been reported that PPARγ agonists exert an antidepressant-like effect via the nitrergic system down regulation (Sadaghiani et al., 2011).

According to the mentioned above, this study was aimed to investigate the antidepressant-like effect of simvastatin and the possible involvement of the NO-cGMP-K_{ATP} channels pathway and PPARγ receptors in mice using forced swimming test (FST). Moreover, the present study was undertaken to characterize the possible involvement of the NO pathway in the interaction of simvastatin with the antidepressant-like activity of fluoxetine.

2. Materials and methods

2.1. Animals

We used male NMRI mice weighing 25–30 g (Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran). Mice had access to chow food and water ad libitum with a light/dark cycle of 12 h, at a temperature of 22 ± 2 °C and 80% humidity. All procedures were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publications No. 80-23, revised 1978) and approved by the local ethics committee at Dezful University of Medical Sciences (IR.DUMS.REC.1396.26). Moreover, we tried to diminish animal suffering as well as to use the minimal number of mice necessary to obtain reliable scientific data.

2.2. Drugs

The following drugs were used: Simvastatin (Sobhan Daru, Tehran, Iran); pioglitazone, a PPARγ agonist (Osveh Company, Tehran, Iran); GW9662, a PPARγ antagonist (Tocris Bioscience, Bristol, UK); N(G)-nitro-L-argininemethyl ester (L-NAME), a non-specific NOS inhibitor, L-arginine, a NO precursor and diazoxide (an ATP-sensitive K^+ channel opener) (Sigma, St. Louis, MO, USA). Methylene blue (NOS and guanylyl cyclase inhibitor) was provided by Merck (Germany). Glibenclamide (ATP-sensitive K^+ channel inhibitor) and fluoxetine were kindly donated by Darupakhsh and Abidi Pharmaceutical Co. (Tehran, Iran), respectively. All drugs were freshly diluted in physiological saline except glibenclamide, pioglitazone, and GW9662 which were dissolved in 1% dimethyl sulfoxide (DMSO) (Fermentas Life Sciences, Lithuania) and then were diluted up to 10 times of the primary volume with normal saline. All drugs were administered intraperitoneally (i.p.) in a constant volume of 10 ml/kg body weight.

2.3. Open field test (OFT)

The locomotor activity of mice was assessed in OFT. This test carried out immediately prior to the forced swimming test to ensure that alterations in motor activity are not responsible for the changes in duration of immobility time, based on the previous report (Stanford, 2007). The apparatus consisted of a wooden box measuring 40 cm × 60 cm × 50 cm. The bottom of the arena was divided into 12 equal squares. Each mouse was placed in the left corner of the field and allowed to explore freely, the number of squares crossing with all paws was counted for 6 min. The test was done under dim light to avoid anxiety behavior. After each test, the apparatus was cleaned with a solution of 10% ethanol to hide animals' clues.

2.4. Forced swim test (FST)

Despair behavior of mice was assessed by this test. Mice were placed in an open cylinder (diameter 10 cm, height 25 cm), containing 19 cm

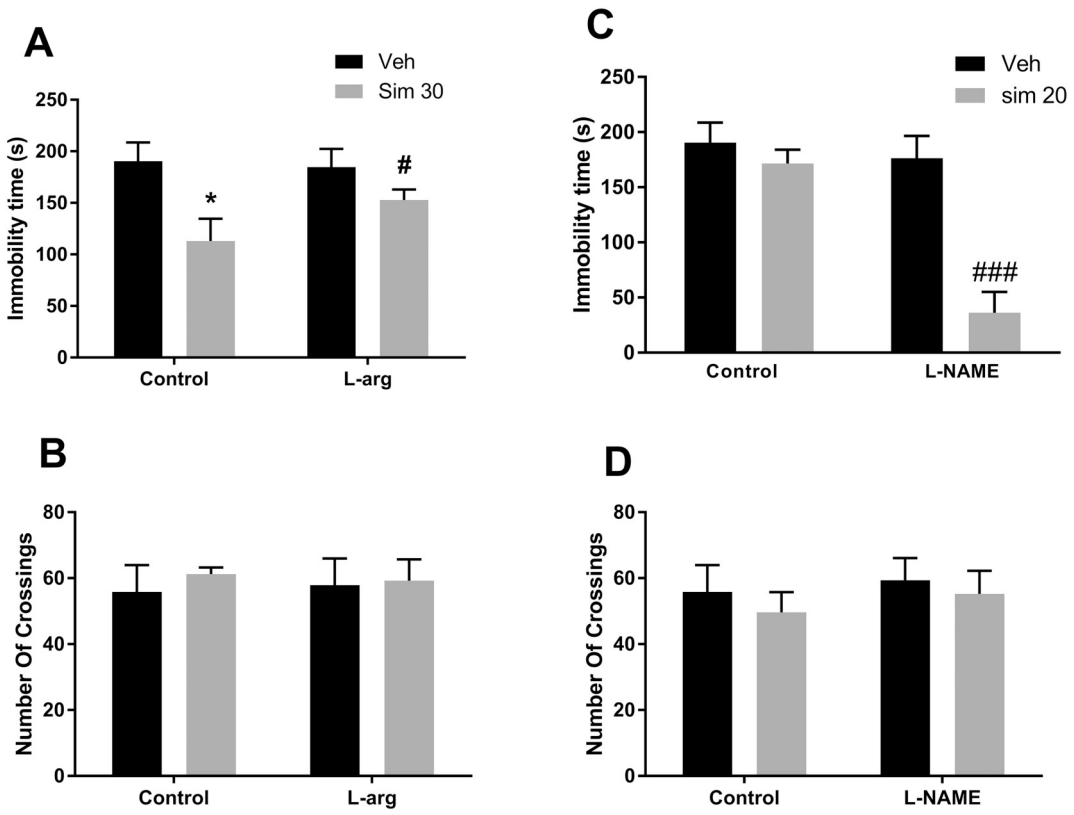

Fig. 2. Evaluation of the involvement of L-arginine–NO pathway in the simvastatin antidepressant-like effect in FST and locomotor activity in the open-field test. The effect of pretreatment with L-arginine (750 mg/kg, i.p.) on the anti-immobility effect of simvastatin (30 mg/kg, i.p.) in the FST and on the number of line crossings in the OFT is shown in panels A and B, respectively. The effect of L-NAME (10 mg/kg, i.p.) in combination with simvastatin (20 mg/kg, i.p.) in the FST and OFT in mice (panels C and D, respectively). Values are expressed as mean ± S.E.M. (n = 6–10). *P < 0.05 compared with the vehicle-treated control group. $^{\#}$P < 0.05 and $^{\#\#\#}$P < 0.001 compared with Sim-treated group, as determined by two-way ANOVA followed by Bonferroni's test.

of water at temperature of 25 ± 1 °C, and were allowed to swim for 6 min. Then, blinded experimenters recorded the period of immobility during the last 4 min of the test. Mice were judged immobile when they stopped struggling and remained floating motionless in the water, making only those movements essential to keep their head above water (Porsolt et al., 1977).

2.5. Treatments

The first series of experiments attempted to investigate the anti-depressant-like effect of simvastatin using FST. Simvastatin at doses of 20, 30, and 40 mg/kg (i.p.) or 10 ml/kg vehicle were administered 30 min prior to the test. Fluoxetine (20 mg/kg, i.p.) was used in FST as a positive control.

To investigate the possible involvement of the L-arginine-NO-cGMP-K_{ATP} pathway in the anti-immobility effects of simvastatin, mice were pre-treated with L-arginine, a nitric oxide precursor (750 mg/kg, i.p., a dose that produces no effect in the FST) or vehicle 30 min prior to simvastatin (30 mg/kg, i.p.) injection. Then, after 30 min the tests were done. In another set of experiments, we investigated the effect of the combined administration of a sub-effective dose of simvastatin (20 mg/kg, i.p.) with a sub-effective dose of L-NAME (10 mg/kg, i.p., a non-selective NO synthase inhibitor) (Shahsavarian et al., 2014).

For analysis the role of cyclic GMP (cGMP) in the antidepressant action of simvastatin, mice received an injection of methylene blue (20 mg/kg, i.p., an inhibitor of soluble guanylate cyclase) or vehicle 30 min before simvastatin (20 mg/kg, i.p.) and then FST was carried out. In addition, we assessed the effect of a combination of an effective dose of simvastatin (30 mg/kg, i.p.) with a sub-effective dose of sildenafil (5 mg/kg, i.p., a PDE-5 inhibitor) (Ludka et al., 2013).

To evaluate the hypothesis that the effect of simvastatin is mediated through the inhibition of K^+ channels, animals were pre-treated with a sub-effective dose of glibenclamide (an ATP-sensitive K^+ channel blocker, 1 mg/kg, i.p.), and 30 min later they received sub-effective doses of simvastatin (20 mg/kg, i.p.). Also, mice were pre-treated with diazoxide (an ATP-sensitive K^+ channel opener, 5 mg/kg, i.p.), and 30 min later they received simvastatin (30 mg/kg, i.p.). Thirty minutes later the mice were submitted to OFT and FST (Ostadhadi et al., 2017).

In another experiment, we assessed whether the anti-immobility effect of simvastatin was produced through the PPARγ receptors. Mice were pre-treated with a sub-effective dose of pioglitazone (a PPARγ receptor agonist, 5 mg/kg, i.p.), and 30 min later they received sub-effective doses of simvastatin (20 mg/kg, i.p.). In addition, we administered GW9662 (a PPARγ receptor antagonist, 2 mg/kg, i.p.) and 30 min later simvastatin (30 mg/kg, i.p.) or vehicle. Doses of simvastatin and other drugs used for mechanism analyses were chosen based on previous reports and studies from our group (Budni et al., 2007; Mansouri et al., 2015; Shahsavarian et al., 2014).

The third set of experiments attempted to evaluate the interaction of simvastatin and fluoxetine in FST, and to elucidate its underlying mechanism. First, we assessed the antidepressant-like effects of fluoxetine to determine its sub-effective dose. For this purpose, fluoxetine (1, 5, and 20 mg/kg, i.p.) was administered 30 min before FST and the corresponding control group received normal saline. Then, combination of sub-effective doses of fluoxetine and simvastatin were administered 30 min prior to the test. Moreover, to investigate the role NO pathway

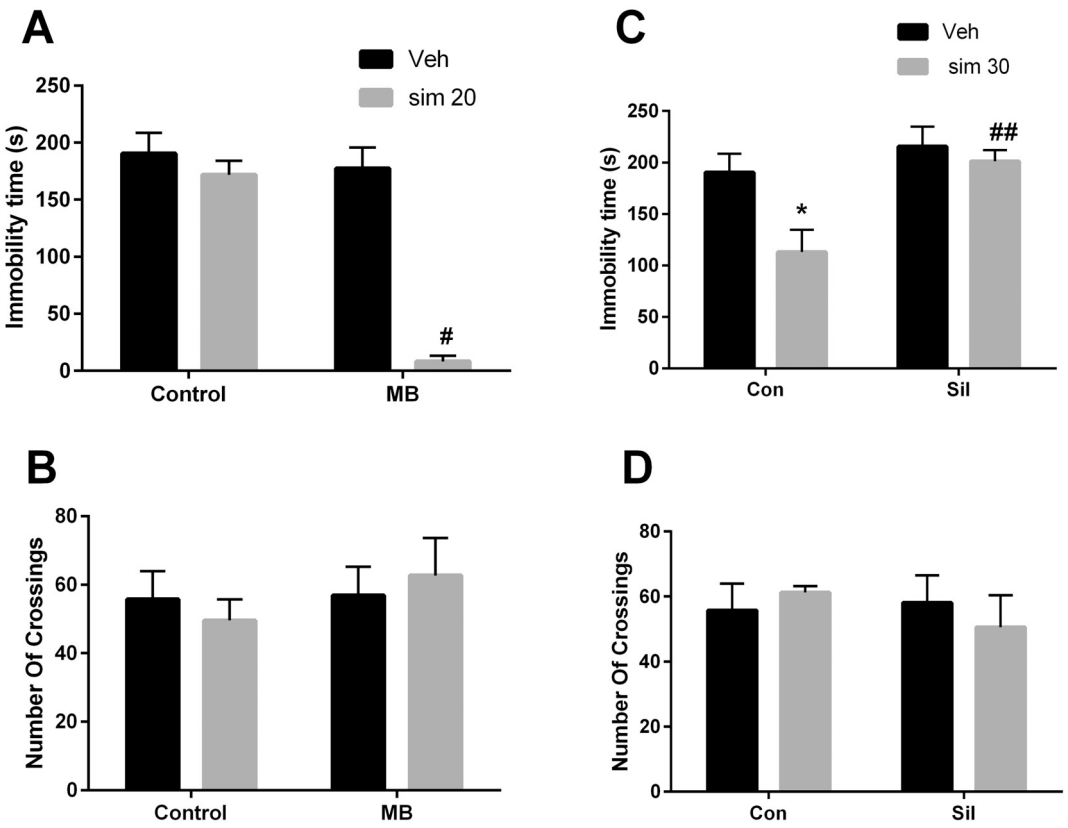

Fig. 3. Evaluation of the involvement of cGMP in the simvastatin (Sim) antidepressant-like effect in FST and locomotor activity in the open-field test. The effect of pretreatment with methylene blue (20 mg/kg, i.p.) on the anti-immobility effect of simvastatin (20 mg/kg, i.p.) in the FST and on the number of line crossings in the OFT is shown in panels A and B, respectively. The effect of sildenafil (5 mg/kg, i.p.) in combination with simvastatin (30 mg/kg, i.p.) in the FST and OFT in mice (panels C and D, respectively). Values are expressed as mean ± S.E.M. (n = 6–10). *P < 0.05 compared with the vehicle-treated control group. ##P < 0.01 compared with Sim-treated group, as determined by two-way ANOVA followed by Bonferroni's test.

in the combination therapy, the NOS inhibitor, L-NAME (10 mg/kg, i.p.) and the NO precursor, L-arginine (750 mg/kg, i.p.) were injected 15 min prior to fluoxetine plus simvastatin.

2.6. Statistical analysis

All data were expressed as mean ± S.E.M. and analyzed using GraphPad software (GraphPad Prism 7.05, San Diego, California, USA). Differences within experimental groups in immobility time and locomotor activity were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test, whereas the interaction between simvastatin and the corresponding interventions were analyzed by two-way ANOVA followed by Bonferroni's post-test. $P < 0.05$ was considered statistically significant in all experiments.

3. Results

3.1. Effect of simvastatin on forced-swimming test and locomotor activity

Anti-immobility effect of simvastatin (20, 30, and 40 mg/kg, i.p.), was determined in Fig. 1. Simvastatin showed significant anti-immobility effects at the doses of 30 mg/kg and 40 mg/kg ($F(4, 26) = 5.15$, $P = 0.003$; Fig. 1A), as the same as fluoxetine at 20 mg/kg. Moreover, simvastatin had no effect on locomotor activity ($F(4, 19) = 0.35$, $P = 0.84$; Fig. 1B).

3.2. Involvement of L-arginine-NO pathway in the simvastatin antidepressant-like effect and locomotor activity

Fig. 2A shows that pre-treatment with L-arginine (750 mg/kg, i.p.) prevented the antidepressant-like effect of simvastatin (30 mg/kg, i.p.) in the FST ($F(1, 23) = 7.72$, $P = 0.01$). Administration of L-arginine alone or in combination with simvastatin did not affect the ambulation in the open-field test ($F(1, 17) = 0.17$, $P = 0.68$, Fig. 2B). The administration of L-NAME (30 mg/kg, i.p) in combination with simvastatin (20 mg/kg, i.p.) produced an antidepressant-like effect compared to either drugs alone, as illustrated in Fig. 2C ($F(1, 23) = 18.58$, $P = 0.0003$). Fig. 2D shows that the administration of L-NAME alone or in combination with simvastatin did not affect locomotor activity in the open-field test ($F(1, 18) = 0.47$, $P = 0.49$).

3.3. Involvement of guanylate cyclase (GC) and cGMP in the simvastatin antidepressant-like effect and locomotor activity

In order to observe the involvement of GC in the antidepressant-like effect induced by the simvastatin, methylene blue (20 mg/kg, i.p.) was used in combination with simvastatin (20 mg/kg, i.p.). As depicted in Fig. 3A, methylene blue in combination with a sub-effective dose of simvastatin also produced an anti-immobility effect in the FST compared to either drug alone ($F(1, 20) = 33.94$, $P < 0.001$). The administration of methylene blue (20 mg/kg, i.p.) alone, or in combination with simvastatin, did not affect the ambulation in the open-field test ($F(1, 16) = 0.36$, $P = 0.55$, Fig. 3B).

Moreover, the anti-immobility effect of simvastatin (30 mg/kg, i.p.)

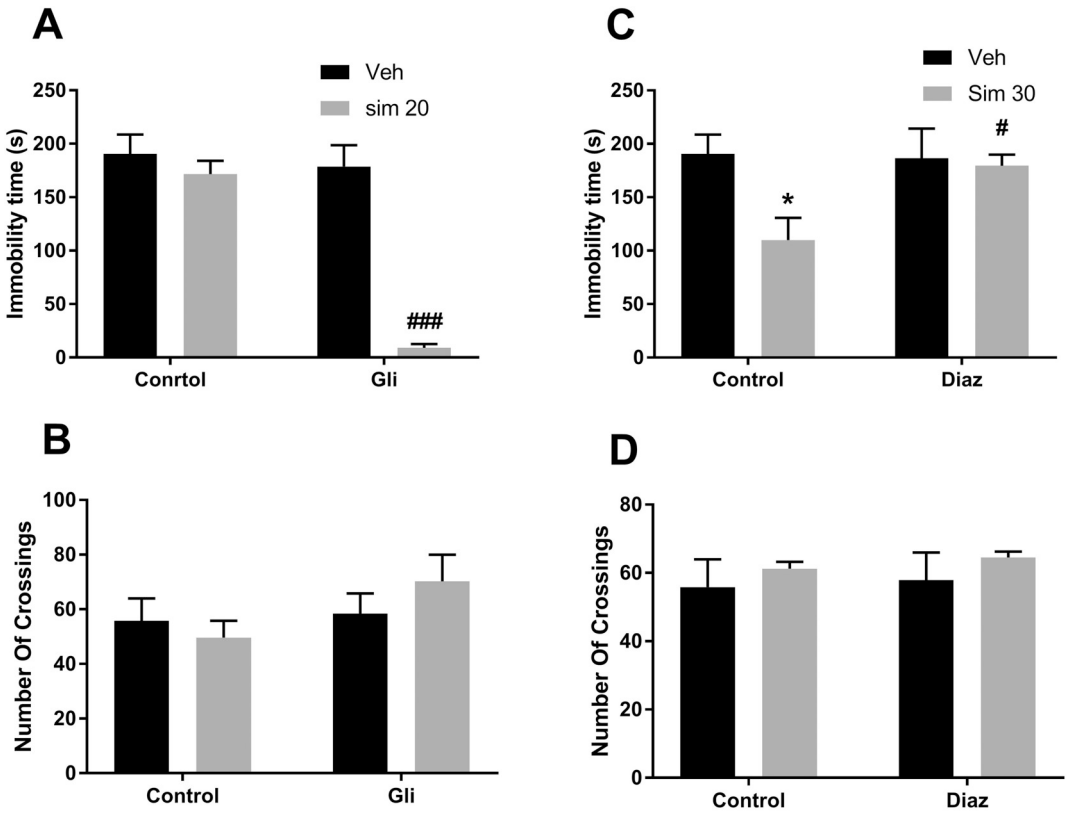

Fig. 4. Evaluation of the involvement of K_{ATP} channels in the simvastatin (Sim) antidepressant-like effect in FST and locomotor activity in the open-field test. The effect of pretreatment with glibenclamide (1 mg/kg, i.p.) on the anti-immobility effect of simvastatin (20 mg/kg, i.p.) in the FST and the number of line crossings in the OFT is shown in panels A and B, respectively. The effect of diazoxide (10 mg/kg, i.p.) in combination with simvastatin (30 mg/kg, i.p.) in the FST and OFT in mice (panels C and D, respectively). Values are expressed as mean ± S.E.M. (n = 6–10). *P < 0.05 compared with the vehicle-treated control group. #P < 0.05 compared with Sim-treated group, as determined by two-way ANOVA followed by Bonferroni's test.

was completely prevented by the pre-treatment with sildenafil (5 mg/kg, i.p.) as shown in Fig. 3C ($F(1, 25) = 7.74$, $P = 0.01$). The administration of sildenafil (5 mg/kg, i.p.) alone or in combination with simvastatin did not affect the locomotor activity in the open-field test ($F(1, 18) = 0.53$, $P = 0.47$, Fig. 3D).

3.4. Involvement of K_{ATP} channels in the simvastatin antidepressant-like effect and locomotor activity

In order to explore the involvement of K_{ATP} channels in the antidepressant-like effect induced by simvastatin, glibenclamide (1 mg/kg, i.p.) was used in combination with simvastatin (20 mg/kg, i.p.). As depicted in Fig. 4A, glibenclamide in combination with a sub-effective dose of simvastatin produced an anti-immobility effect compared to either drug alone in the FST ($F(1, 23) = 21.2$, $P = 0.0001$). In addition, glibenclamide at this dose showed no effect on the locomotor activity of the animals in OFT ($F(1, 20) = 1.16$, $P = 0.29$, Fig. 4B).

Moreover, as depicted in Fig. 4C, the anti-immobility effect of simvastatin (30 mg/kg, i.p.) was completely prevented by the pre-treatment of mice with diazoxide (10 mg/kg, i.p., a K_{ATP} channel opener) ($F(1, 25) = 4.37$, $P = 0.04$). Additionally, diazoxide at this dose showed no effect on locomotor activity of the animals in OFT ($F(1, 17) = 0.005$, $P = 0.94$, Fig. 4D).

3.5. Involvement of PPARγ receptors in the simvastatin antidepressant-like effect and locomotor activity

To investigate the role of PPARγ in antidepressant-like effect of simvastatin, pioglitazone (5 mg/kg, i.p.) was used in combination with simvastatin (20 mg/kg, i.p.). As depicted in Fig. 4A, pioglitazone in combination with a sub-effective dose of simvastatin produced an anti-immobility effect compared to either drug alone in the FST ($F(1, 23) = 5.49$, $P = 0.02$). In addition, pioglitazone at this dose showed no effect on locomotor activity of the animals in OFT ($F(1, 20) = 0.12$, $P = 0.72$, Fig. 4B).

Moreover, it was observed that GW9662 significantly reversed the antidepressant-like effect of simvastatin in FST ($F(1, 22) = 5.09$, $P = 0.03$; Fig. 5C). Administration of GW9662 alone or in combination with simvastatin did not alter the locomotor activity ($F(1, 19) = 0.25$, $P = 0.62$; Fig. 5D).

3.6. Effect of fluoxetine and its combination with simvastatin on FST and locomotor activity

Antidepressant-like effects of fluoxetine (1, 5, and 20 mg/kg, i.p.), administered 30 min before FST, has been shown in Fig. 6A. Data showed that fluoxetine significantly reduced immobility time at doses 5 and 20 mg/kg, while 1 mg/kg did not show significant effect ($F(3, 26) = 6.68$, $P = 0.002$; Fig. 6A). However, fluoxetine did not alter locomotor activity ($F(3, 23) = 0.21$, $P = 0.88$; Fig. 6B). Moreover, results revealed that co-administration of sub-effective doses of simvastatin (20 mg/kg) and fluoxetine (1 mg/kg) significantly induced an antidepressant-like effect in FST ($F(1, 20) = 5.96$, $P = 0.02$; Fig. 6C). The locomotor activity did not alter as compared to the corresponding control groups ($F(1, 20) = 0.13$, $P = 0.71$; Fig. 6D).

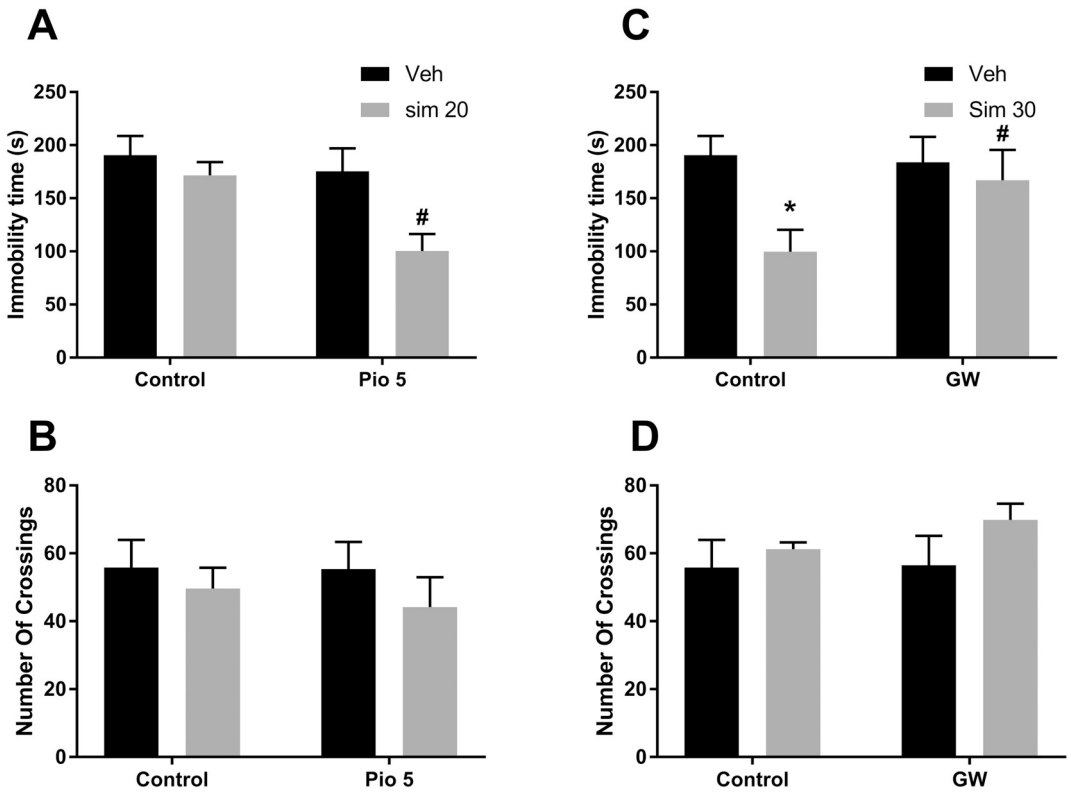

Fig. 5. Evaluation of the involvement of PPARγ receptors in the simvastatin (Sim) antidepressant-like effect in FST and locomotor activity in the open-field test. The effect of pretreatment with pioglitazone (Pio, 5 mg/kg, i.p.) on the anti-immobility effect of simvastatin (20 mg/kg, i.p.) in the FST and the number of line crossings in the OFT is shown in panels A and B, respectively. The effect of GW-9662 (2 mg/kg, i.p.) in combination with simvastatin (30 mg/kg, i.p.) in the FST and OFT in mice (panels C and D, respectively). Values are expressed as mean ± S.E.M. (n = 6–10). *P < 0.05 compared with the vehicle-treated control group. #P < 0.05 compared with Sim-treated group, as determined by two-way ANOVA followed by Bonferroni's test.

3.7. Involvement of the L-arginine-NO pathway on the anti-immobility effect of fluoxetine plus simvastatin on FST and locomotor activity

As shown in Fig. 7A, administration of L-arginine (750 mg/kg, i.p.) antagonized the antidepressant-like effect of combination of simvastatin and fluoxetine ($F(1,21) = 18.53$, $P = 0.0003$; Fig. 7A). On the other hand, the antidepressant-like effect of the combination of simvastatin plus fluoxetine was potentiated by L-NAME ($F(1, 22) = 58.29$, $P < 0.0001$; Fig. 7C). In addition, administration of L-arginine or L-NAME with simvastatin and fluoxetine did not alter the locomotor activity in the open-field test (Fig. 7B and D).

4. Discussion

Our results showed that intraperitoneal simvastatin could significantly produce an antidepressant-like effect in the forced swimming test (FST) through modulating the L-arginine-NO-cGMP-K_{ATP} pathway and PPARγ receptors without modifying motor performance in mice. Furthermore, simvastatin could potentiate the antidepressant-like activity of fluoxetine through inhibition of the NO pathway.

Statins have been widely used to improve macular degeneration, dementia, stroke, and depression. However, the association between statins and the depressive mood is steel unclear (Raja and Dreyfus, 2004; You et al., 2013). Some studies showed that higher cholesterol concentration was associated with signs of depression (Nakao and Yano, 2004; Tyrovolas et al., 2009). Furthermore, literature has indicated the effectiveness of statins in these disorders beyond their indication for lipid-lowering drugs (Tsai, 2007).

In agreement with the previous studies, our results illustrated simvastatin administration produced a dose-dependent reduction in the immobility time in FST, which indicates its antidepressant-like effect. Moreover, our data indicated that simvastatin could potentiate the antidepressant-like activity of fluoxetine in mice. In this regard, it has been demonstrated that simvastatin can augment the antidepressant effects of fluoxetine and imipramine in rats (Patke et al., 2015; Lin et al., 2014). Further, we showed that the role of NO pathway in this synergistic effect.

Nitric oxide (NO) is synthesized in brain by NO synthase (NOS) from the amino-acid L-arginine. Literature implicated that NO has a neuromodulatory role in many physiological and pathological conditions in CNS including stroke, epilepsy, neurotoxicity, neurodegenerative diseases and depression (da Silva et al., 2000). Many studies have pointed to L-arginine-NO-cGMP modulation by drugs with antidepressant-like properties (Morreti et al., 2011; Zomkowski et al., 2010).

In the present study, pretreatment with L-arginine (NOS substrate) was able to reverse the reduction of immobility time induced by simvastatin in FST. The role of the L-arginine-NO pathway in the simvastatin anti-depressant effect in FST is reinforced by the finding that a sub-effective dose of L-NAME produced an antidepressant-like effect in combination with a sub-effective dose of simvastatin. Interestingly, the same results observed in the combination therapy of simvastatin and fluoxetine suggesting the inhibitory role of NO pathway in the antidepressant-like effect of simvastatin alone or in combination with fluoxetine. In this regard, Fossier et al. (1999) indicated that NO transforms serotonin (as an antidepressant neurotransmitter) into an inactive form and this affects neuromodulation. They showed that this modulatory effect of serotonin was reduced not only in the presence of a NO donor but also when endogenous NO synthase was activated.

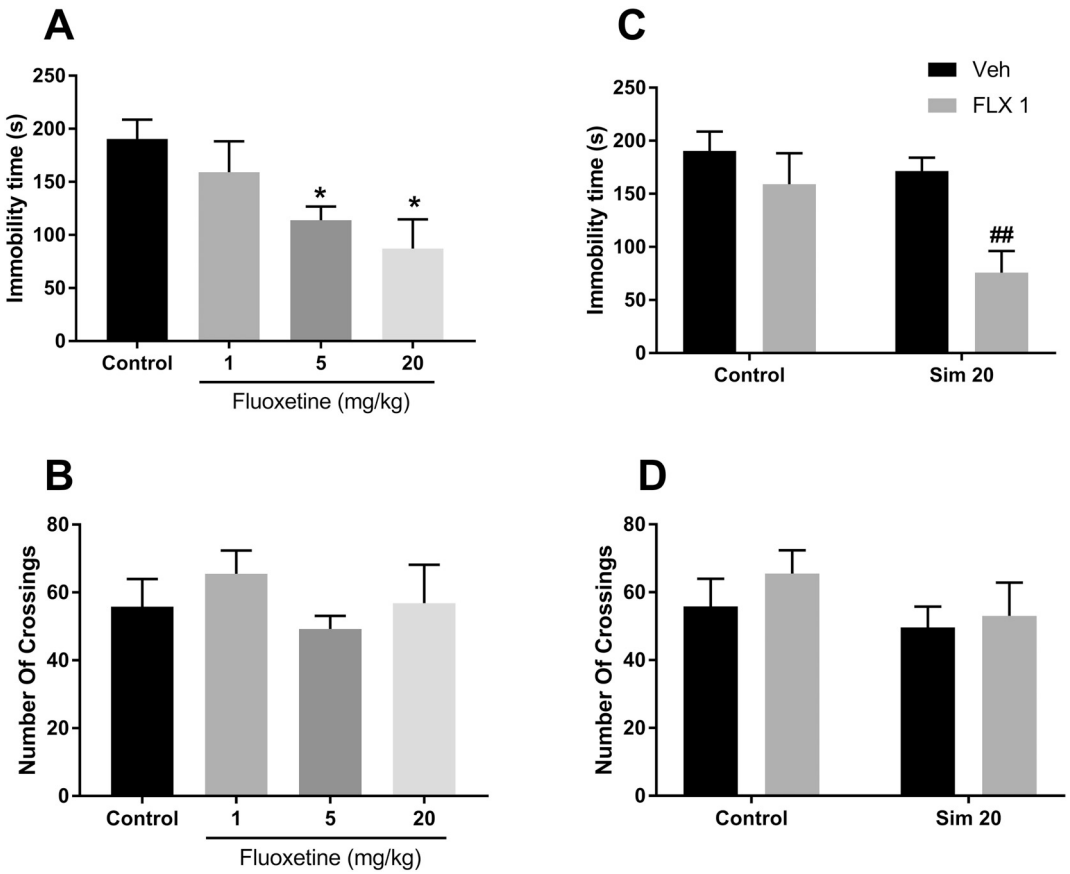

Fig. 6. Effect of administration of fluoxetine (FLX) in the immobility time in the FST (A) and the number of line crossing in the open-field test (B), administered 30 min prior to the test. The effect of simvastatin (20 mg/kg, i.p.) in combination with fluoxetine (1 mg/kg, i.p.) in the FST and OFT in mice (panels C and D, respectively). Values are expressed as mean ± S.E.M. (n = 6–10). *P < 0.05 compared with the vehicle-treated control group (one-way ANOVA followed by Tukey's test). #P < 0.05 compared with FLX-treated group (two-way ANOVA followed by Bonferroni's test).

In addition, a sub-effective dose of methylene blue (inhibitor of both NOS and sGC) produced an antidepressant-like effect when co-injected with a sub-effective dose of simvastatin. Furthermore, sildenafil, as a selective PDE5 inhibitors, prevented the simvastatin antidepressant-like effect in FST. Thus, simvastatin antidepressant-like effect may be mediated through the reduction of cGMP, as a consequence of NO synthesis reduction. The results obtained here reveal the assumption that the antidepressant-like effect of simvastatin involves the L-arginine-NO-cGMP pathway. In this regard, Shahsavarian et al. (2014) reported that another statin drug, atorvastatin, produced antidepressant-like effect via NO pathway (Shahsavarian et al., 2014).

Previous studies suggested that elevation the levels of NO and cGMP can stimulate K_{ATP} channels. So, the blocking of the NO signaling system diminishes the NO concentrations and finally leads to the blockade of these channels (Kaster et al., 2005). The blockers of these K^+ channels were reported to exert an antidepressant-like effect in the FST at higher doses than those used in the present study (Kaster et al., 2007). Our findings showed that administration of glibenclamide (a K_{ATP} channel blocker) could potentiate the effect of a sub-effective dose of simvastatin. On the other hand, diazoxide as a K_{ATP} channel opener reversed the anti-immobility time provoked by an effective dose of simvastatin in mice. In agreement with our results, the antidepressant-like properties of various antidepressants including fluoxetine, paroxetine, amitriptyline and desipramine in the FST were reversed by K_{ATP} channel agonists (Choi et al., 2004; Cryan et al., 2002). Also, several studies have shown that the combined administration of antidepressants and K^+ channel blockers produced an antidepressant-like effect in the FST in mice (Bortolatto et al., 2010; Kaster et al., 2007).

The presence of PPARγ receptors in some areas of brains such as hypothalamus, pituitary nuclei, hippocampus, basal ganglia and frontal cortex along with the fact that these areas play an important role in the depression made scientists to consider the role of these receptors on depressive mood (Inestrosa et al., 2005). The antidepressant-like effects of PPARγ receptors agonist were first observed in severe unresponsive depression (Kemp et al., 2009). On the other hand, literature implicated some properties of simvastatin are probably PPARγ-mediated (Corti et al., 2004; Mansouri et al., 2017). In the present work, we indicated that simvastatin reduced depression-like behaviors in mice when administered with pioglitazone, a PPARγ receptor agonist. While, GW9662 (a PPARγ receptor antagonist) attenuated the antidepressant-like effect of simvastatin. Thus, it can be concluded that an increase in PPARγ activity plays a role in the antidepressant-like effect of simvastatin in the FST. In this regard, Shahsavarian et al. (2014) showed that the antidepressant-like effect of atorvastatin is mediated at least in part through PPARγ receptors (Shahsavarian et al., 2014).

5. Conclusion

In conclusion, the present study reported the antidepressant-like activity of simvastatin in the mouse forced swiming test. Moreover, we have shown for the first time that the antidepressant-like activity of simvastatin is probably mediated through NO-cGMP-K_{ATP} signaling system and PPARγ receptors. Also, the antidepressant-like effects of simvastatin could not be attributed to an increase in locomotor activity.

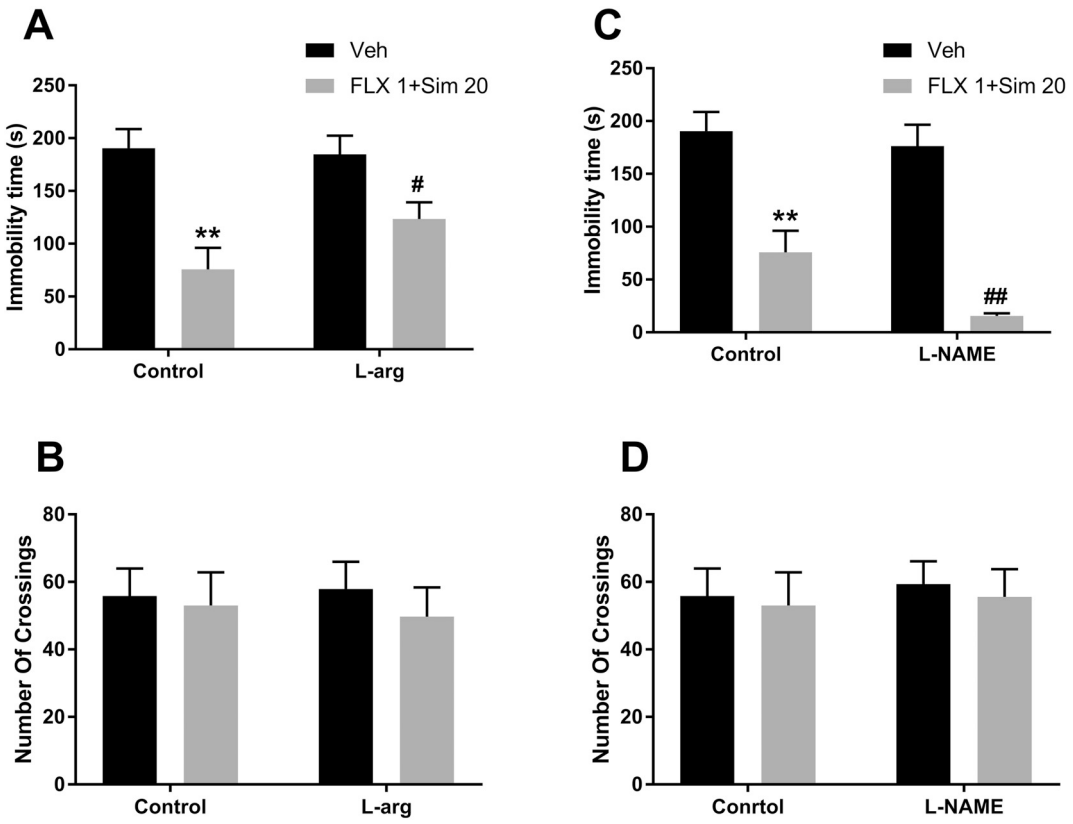

Fig. 7. Evaluation of the involvement of the L-arginine-NO pathway in the combination of simvastatin and fluoxetine antidepressant-like effect in FST and locomotor activity in the open-field test. The effect of pretreatment with L-arginine (750 mg/kg, i.p.) on the anti-immobility effect of concurrent simvastatin (20 mg/kg, i.p.) and fluoxetine (1 mg/kg, i.p.) in the FST and on the number of line crossings in the OFT is shown in panels A and B, respectively. The effect of L-NAME (10 mg/kg, i.p.) in combination with concurrent simvastatin and fluoxetine in the FST and OFT in mice (panels C and D, respectively). Values are expressed as mean ± S.E.M. (n = 6–10). **P < 0.01 compared with the vehicle-treated control group. $^{\#}$P < 0.05 and $^{\#\#}$P < 0.01 compared with FLX + Sim-treated group, as determined by two-way ANOVA followed by Bonferroni's test.

Moreover, simvastatin could potentiate the antidepressant-like activity of fluoxetine in a synergistic manner through the NO pathway.

Acknowledgments

This paper is issued from the medical thesis of (Reza Naserzadeh and Niloofar Abad) and financial support was provided by the Vice Chancellor of Research, Dezful University of Medical Sciences (IR.DUMS.REC.1396.26), Dezful, Iran. Also, The present study was supported by a grant funded by Dezful University of Medical Sciences.

Conflict of interest

The authors declare that there is no conflict of interests.

References

Bortolatto, C.F., Jesse, C.R., Wilhelm, E.A., Nogueira, C.W., 2010. Involvement of potassium channels in the antidepressant-like effect of venlafaxine in mice. Life Sci. 86 (9–10), 372–376.
Budni, J., Gadotti, V.M., Kaster, M.P., Santos, A.R., Rodrigues, A.L.S., 2007. Role of different types of potassium channels in the antidepressant-like effect of agmatine in the mouse forced swimming test. Eur. J. Pharmacol. 575, 87–93.
Choi, J.S., Choi, B.H., Ahn, H.S., Kim, M.J., Han, T.H., Rhie, D.J., 2004. Fluoxetine inhibits A-type potassium currents in primary cultured rat hippocampal neurons. Brain Res. 1018, 201–207.
Cimini, A., Benedetti, E., Cristiano, L., Sebastiani, P., D'Amico, M.A., D'Angelo, B., Di Loreto, S., 2005. Expression of peroxisome proliferator-activated receptors (PPARs) and retinoic acid receptors (RXRs) in rat cortical neurons. Neuroscience 130 (2), 325–337.
Corti, R., Osende, J.I., Fallon, J.T., Fuster, V., Mizsei, G., Jneid, H., Wright, S.D., Chaplin, W.F., Badimon, J.J., 2004. The selective peroxisomal proliferator- activated receptor-gamma agonist has an additive effect on plaque regression in combination with simvastatin in experimental atherosclerosis: invivostudy by high-resolution magnetic resonance imaging. J. Am. Coll. Cardiol. 43 (3), 464–473.
Cryan, J.F., Markou, A., Lucki, I., 2002. Assessing antidepressant activity in rodents: recent developments and future needs. Trends Pharmacol. Sci. 23 (5), 238–245.
da Silva, G.D., Matteussi, A.S., dos Santos, A.R., Calixto, J.B., Rodrigues, A.L., 2000. Evidence for dual effects of nitric oxide in the forced swimming test and in the tail suspension test in mice. Neuroreport 11 (17), 3699–3702.
Dietschy, J.M., Turley, S.D., 2001. Cholesterol metabolism in the brain. Curr. Opin. Lipidol. 12, 105–112.
ElBatsh, M.M., 2015. Antidepressant-like effect of simvastatin in diabetic rats. Can. J. Physiol. Pharmacol. 93 (8), 649–656.
Esplugues, J.V., 2002. NO as a signalling molecule in the nervous system. Br. J. Pharmacol. 135 (5), 1079–1095.
Feinstein, D.L., Galea, E., Gavrilyuk, V., Brosnan, C.F., Whitacre, C.C., Dumitrescu-Ozimek, L., Landreth, G.E., Pershadsingh, H.A., Weinberg, G., Heneka, M.T., 2002. Peroxisome proliferator-activated receptor-gamma agonists prevent experimental autoimmune encephalomyelitis. Ann. Neurol. 51 (6), 694–702.
Fossier, P., Blanchard, B., Ducrocq, C., Leprince, C., Tauc, L., Baux, G., 1999. Nitric oxide transforms serotonin into an inactive form and this affects neuromodulation. Neuroscience 93 (2), 597–603.
Ghorbanzadeh, B., Mansouri, M.T., Naghizadeh, B., Alboghobeish, S., 2018. Local anti-nociceptive action of fluoxetine in the rat formalin assay: role of L-arginine/nitric oxide/cGMP/KATP channel pathway. Can. J. Physiol. Pharmacol. 96 (2), 165–172.
Inestrosa, N.C., Godoy, J.A., Quintanilla, R.A., Koenig, C.S., Bronfman, M., 2005. Peroxisome proliferator-activated receptor gamma is expressed in hippocampal neurons and its activation prevents beta-amyloid neurodegeneration: role of Wnt signaling. Exp. Cell Res. 304, 91–104.
Jeong, S.Y., Ha, T.S., Park, C.S., Uhm, D.Y., Chung, S., 2001. Nitric oxide directly activates large conductance Ca^{2+}-activated K^{+} channels (rSlo). Mol. Cell (12), 97–102 2001.
Kaster, M.P., Ferreira, P.K., Santos, A.R.S., Rodrigues, A.L.S., 2005. Effects of potassium channel inhibitors in the forced swimming test: possible involvement of L-arginine-nitric oxidesoluble guanylate cyclase pathway. Behav. Brain Res. 165, 204–209.
Kaster, M.P., Budni, J., Binfare, R.W., Santos, A.R.S., Rodrigues, A.L.S., 2007. The inhibition of different types of potassium channels underlies the antidepressant-like effect of adenosine in the mouse forced swimming test. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 31, 690–696.
Kay, J., Atiq, R., 2006. Treatment-resistant depression. Psychiatry 10 (Part 1), 1–11.

Kemp, D.E., Ismail-Beigi, F., Calabrese, J.R., 2009. Antidepressant response associated with pioglitazone: support for an overlapping pathophysiology between major depression and metabolic syndrome. Am. J. Psychiatry 166 (5), 619.
Lin, P.Y., Chang, A.Y., Lin, T.K., 2014. Simvastatin treatment exerts antidepressant-like effect in rats exposed to chronic mild stress. Pharmacol. Biochem. Behav. 124, 174–179.
Lu, D., Mahmood, A., Qu, C., Goussev, A., Lu, M., Chopp, M., 2004. Atorvastatin reduction of intracranial hematoma volume in rats subjected to controlled cortical impact. J. Neurosurg. 101 (5), 822–825.
Ludka, F.K., Zomkowski, A.D., Cunha, M.P., Dal-Cim, T., Zeni, A.L., Rodrigues, A.L., Tasca, C.I., 2013. Acute atorvastatin treatment exerts antidepressant-like effect in mice via the L-arginine–nitric oxide–cyclic guanosine monophosphate pathway and increases BDNF levels. Eur. Neuropsychopharmacol. 23 (5), 400–412.
Mansouri, M.T., Khodayar, M.J., Tabatabaee, A., Ghorbanzadeh, B., Naghizadeh, B., 2015. Modulation of morphine antinociceptive tolerance and physical dependence by co-administration of simvastatin. Pharmacol. Biochem. Behav. 137, 38–43.
Mansouri, M.T., Naghizadeh, B., Ghorbanzadeh, B., Alboghobeish, S., 2017. Systemic and local anti-nociceptive effects of simvastatin in the rat formalin assay: role of peroxisome proliferator-activated receptor gamma and nitric oxide. J. Neurosci. Res. 95, 1776–1785.
Morreti, M., Freitas, A.E., Budni, J., Fernandes, S.C., Balen, G., de, O., Rodrigues, A.L.S., 2011. Involvement of nitric oxide– cGMP pathway in the antidepressant-like effect of ascorbic acid in the tail suspension test. Behav. Brain Res. 225, 328–333.
Nakao, M., Yano, E., 2004. Relationship between major depression and high serum cholesterol in Japanesemen. Tohoku J. Exp. Med. 204 (4), 273–287.
Ostadhadi, S., Akbarian, R., Norouzi-Javidan, A., Nikoui, V., Zolfaghari, S., Chamanara, M., Dehpour, A.R., 2017. Possible involvement of ATP-sensitive potassium channels in the antidepressant-like effects of gabapentin in mouse forced swimming test. Can. J. Physiol. Pharmacol. 95 (7), 795–802.
Patke, A., Tripathi, R., Patke, V.G., Sonawane, D., Rege, N., 2015. Antidepressant activity of simvastatin in behavioral models of depression in rats. Int. J. Res. Med. Sci. 3 (7), 1666–1671.
Piermartiri, T.C., Vandresen-Filho, S., de Araujo Herculano, B., Martins, W.C., Dal'agnolo, D., Stroeh, E., Carqueja, C.L., Boeck, C.R., Tasca, C.I., 2009. Atorvastatin prevents hippocampal cell death due to quinolinicacid-induced seizures in mice by increasing Akt phosphorylation and glutamate uptake. Neurotox. Res. 16 (2), 106–115.
Porsolt, R.D., Bertin, A., Jalfre, M., 1977. Behavioral despair in mice: a primary screening test for antidepressants. Arch. Int. Pharmacodyn. Ther. 229 (2), 327–336.
Pucadyil, T.J., Chattopadhyay, A., 2006. Role of cholesterol in the function and organization of Gprotein coupled receptors. Prog. Lipid Res. 45, 295–333.
Raja, S.G., Dreyfus, G.D., 2004. Statins: much more than just a lipid-lowering therapy. Indian Heart J. 56 (3), 204–209.
Renaud, J., Bédard, E., 2013. Depression in the elderly with visual impairment and its association with quality of life. Clin. Interv. Aging 8, 931–943.
Renshaw, P.F., Parsegian, A., Yang, C.K., Novero, A., Yoon, S.J., Lyoo, I.K., Cohen, B.M., Carlezon, W.A., 2009. Lovastatin potentiates the antidepressant efficacy of fluoxetine in rats. Pharmacol. Biochem. Behav. 92 (1), 88–92.
Rosa, A.O., Kaster, M.P., Binfare, R.W., Morales, S., Martin-Aparicio, E., Navarro-Rico, M.L., Martinez, A., Medina, M., García, A.G., López, M.G., Rodrigues, A.L., 2008. Antidepressant-like effect of the novel thiadiazolidinone NP031115 in mice. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 32, 1549–1556.
Sadaghiani, M.S., Javadi-Paydar, M., Gharedaghi, M.H., Fard, Y.Y., Dehpour, A.R., 2011. Antidepressant-like effect of pioglitazone in the forced swimming test in mice: the role of PPAR-gamma receptor and nitric oxide pathway. Behav. Brain Res. 224 (2), 336–343.
Shahsavarian, A., Javadi, S., Jahanabadi, S., Khoshnoodi, M., Shamsaee, J., Shafaroodi, H., Mehr, S.E., Dehpour, A., 2014. Antidepressant-like effect of atorvastatin in the forced swimming test in mice: the role of PPAR-gamma receptor and nitric oxide pathway. Eur. J. Pharmacol. 115 (745), 52–58.
Stanford, S.C., 2007. The open field test: reinventing the wheel. J. Psychopharmacol. 21 (2), 134–136.
Sundararajan, S., Gamboa, J.L., Victor, N.A., Wanderi, E.W., Lust, W.D., Landreth, G.E., 2005. Peroxisome proliferator-activated receptor-gamma ligands reduce inflammation and infarction size in transient focal ischemia. Neuroscience 130 (3), 685–696.
Tsai, S.J., 2007. Statins may enhance the proteolytic cleavage of proBDNF: implications for the treatment of depression. Med. Hypotheses 68 (6), 1296–1299.
Tyrovolas, S., Lionis, C., Zeimbekis, A., Bountziouka, V., Micheli, M., Katsarou, A., Papairakleous, N., Metallinos, G., Makri, K., Polychronopoulos, E., Panagiotakos, D.B., 2009. Increased body mass and depressive symptomatology are associated with hypercholesterolemia, among elderly individuals; results from the MEDIS study. Lipids Health Dis. 8, 10.
Vevera, J., Valeš, K., Fišar, Z., Hroudová, J., Singh, N., Stuchlík, A., Kačer, P., Nekovářová, T., 2016. The effect of prolonged simvastatin application on serotonin uptake, membrane microviscosity and behavioral changes in the animal model. Physiol. Behav. 158, 112–120.
Yildiz, F., Erden, B.F., Ulak, G., Utkan, T., Gacar, N., 2000. Antidepressant-like effect of 7-nitroindazole in the forced swimming test in rats. Psychopharmacology 149 (1), 41–44.
You, H., Lu, W., Zhao, S., Hu, Z., Zhang, J., 2013. The relationship between statins and depression: a review of the literature. Expert. Opin. Pharmacother. 14 (11), 1467–1476.
Zomkowski, A.D.E., Engel, D., Gabilan, N.H., Rodrigues, A.L.S., 2010. Involvement of NMDA receptors and L-arginine–nitric oxide–cyclic guanosine monophosphate pathway in the antidepressant-like effects of escitalopram in the forced swimming test. Eur. Neuropsychopharmacol. 20, 793–801.