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Experimental Research Article

Exploring the antidepressant-like effects of atorvastatin in ovariectomized mice: involvement of the nitric oxide pathway

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ABSTRACT

Introduction: Atorvastatin, a lipid-soluble statin, is commonly used in managing high cholesterol levels and has been demonstrated to possess pleiotropic effects, such as antidepressant and neuroprotective properties. Women are more likely to suffer from depression because hormone levels change during ovariectomy and menopause. However, the role of statins in the treatment of ovariectomy-induced depressive behavior has not been adequately studied. We explored atorvastatin's potential antidepressant effects as well as the potential function of the nitric oxide pathway in ovariectomized (OVX) mice.

Methods: Female mice underwent ovary removal, followed by administration of varying doses of atorvastatin alone or in conjunction with either a non-specific NO synthase inhibitor (L-NAME) or an NO precursor (L-arginine). Behavioral alterations were assessed using the Tail Suspension Test (TST), Forced Swim Test (FST), and Open Field Test (OFT), while hippocampal nitrite levels were also measured.

Results: One week post-procedure, OVX mice displayed a notably longer period of immobility in comparison to the sham group. OVX animals treated with atorvastatin (0.1 and 1 mg/kg) demonstrated antidepressant properties; additionally, OVX mice that received a sub-effective dose of atorvastatin plus a sub-effective dose of L–NAME demonstrated pronounced antidepressant-like effects ($P<0.05$). L-arginine counteracted the antidepressant-like effects of a high dose of atorvastatin in OVX mice but did not affect their levels of locomotor activity in the OFT. Furthermore, atorvastatin administration prevented the increased hippocampal nitrite concentrations caused by ovariectomy ($P<0.05$).

Conclusion: The research revealed that atorvastatin exhibits significant antidepressant properties in OVX mice, potentially by suppressing the nitric oxide pathway.

Keywords:
Ovariectomy
Depression
Atorvastatin
Nitric Oxide
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Introduction

Currently, the prevalence of depression disorder has emerged as a significant issue within society (Kordjazy et al., 2015). Epidemiologic studies suggest that women are more susceptible to the development of depressive symptoms (Saeedi Saravi et al., 2016). Hormonal fluctuations, such as low estrogen levels following ovariectomy, menopause, and postpartum, can cause women to experience episodes of low self-esteem, diminished life satisfaction, and low energy (Avis et al., 1994; Heydarpour et al., 2013). Atorvastatin is categorized as a lipophilic statin known for reducing levels of cholesterol in the blood (Schachter 2005). Statins inhibit the enzyme 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme in cholesterol synthesis. Several studies have shown that statins have pleiotropic effects, which include neuroprotective effects. Atorvastatin has been shown to protect neurons in vivo from glutamatergic excitotoxicity, improve memory, and prevent ischemia-induced cell death in rodent models of Parkinson's and Alzheimer's disease (Taniguti et al., 2019). It can exert a neuroprotective effect by inhibiting TNF-α release, preventing cell death caused by ischemia, and reducing toxicity caused by beta-amyloid (Kumar et al., 2012; Piermartiri et al., 2010; Vandresen-Filho et al., 2013). Additionally, atorvastatin has been found to have an antidepressant-like effect, although there is still limited knowledge regarding its mechanism of action in the central nervous system (CNS) (Young-Xu et al., 2003).

Depression's pathophysiology involves a multitude of neural pathways. Given that nitric oxide (NO) acts as a neurotransmitter in both the central and peripheral nervous systems, it is possible that this gaseous substance may play a part in the development of schizophrenia, epilepsy, and major depressive disorder (MDD) (Akyol et al., 2002; Banach and Piskorska 2012; Maes 1999; Nasyrova et al., 2015). In the presence of O2 and NADPH, three different forms of nitric oxide synthase, namely *iNOS*, *eNOS,* and *nNOS,* convert L-arginine to NO. (da Silva et al., 2000; Esplugues 2002; Nathan and Wen Xie 1994). By modulating the effects of serotonin (5-HT), dopamine (DA), and norepinephrine (NE), NO may play a key role in the neurobiology of depression (Khoshnoodi et al., 2015). nNOS shows significant expression levels in regions such as the cortex, cerebellum, and hippocampus, which are essential for processes like memory, learning, and neurogenesis (Chrapko et al., 2004; Reif et al., 2004). Additionally, it's thought that in bilaterally ovariectomized mice, a decrease in hippocampal NO levels or an inhibition of NOS can produce antidepressant-like effects (Joca and Guimares 2006). Chronic stress has the potential to disrupt the regulation of immunity through the over-excitation of the immune system, resulting in a mild form of inflammation and the induction of inducible NO synthase (iNOS). Therefore, iNOS could have a significant impact on depressive tendencies. The utilization of NOS inhibitors has been proposed as an innovative category of treatments for Major Depressive Disorder (MDD) through the reduction of cyclic guanosine monophosphate (cGMP) and also nitric oxide levels (Khoshnoodi et al., 2015). There is evidence that estrogen acts on the nitrenergic system in the hippocampus. The temporary changes in reproductive hormones throughout the estrous cycle indicate that this phenomenon can take place within normal physiological conditions (Gotti et al., 2009). Estradiol enhances the production of nitric oxide in the hippocampus by upregulating the expression of nNOS through estrogen receptor-β. Therefore, reduced estrogen levels in the female hippocampus may account for the lower levels of nitric oxide compared to the male hippocampus. Additionally, variations in estrogen levels could potentially contribute to the emergence of mood disorders among women. Specifically, rather than estrogen itself, it is nitric oxide (NO) in the hippocampus that acts as a key factor in determining gender disparities in emotional behaviors. This is because hippocampal NO is crucial for the behavioral impact of estrogen (Hu et al., 2012).

The present study focuses on investigating the potential antidepressant properties of atorvastatin in female ovariectomized (OVX) mice through behavioral assessments like the Forced Swim Test (FST) and the Tail Suspension Test (TST). In addition, the study aims to explore how the NO signaling pathway influences atorvastatin's protective effect against mood disorders. Our hypothesis suggests that atorvastatin may, in part, influence behavior in OVX mice via the L-arginine/nitric oxide signaling pathway.

Material and Methods

Animals

In this study, female NMRI *mice* weighing 25-30 g were purchased from Shahid Sadoughi University of

Medical Sciences in Yazd, Iran. The animals were housed in an environment with alternating 12-hour periods of light and darkness, maintained at a temperature between 21 and 23°C. Behavioral *experiments* were conducted in the afternoon (*12:00–18:00).* Animals had complete access to food and water, except in cases where ovariectomy and behavioral testing were performed. Each animal underwent a single ovariectomy and behavioral test with six to eight animals in each group. All tests and procedures were conducted according to the ethical guidelines of the Ethics Committee of Shahid Sadoughi University of Medical Sciences and approved by the Ethics Committee of Shahid Sadoughi University of Medical Sciences (IR.SSU.MEDICINE.REC.1396.90).

Ovariectomy

During the ovariectomy procedure, mice were anesthetized using ketamine and xylazine hydrochloride (50 mg/kg and 5 mg/kg i.p., respectively). The surgical technique was carried out as previously *described* (Kalbasi Anaraki et al., 2008; Sadeghi et al., 2009). The fatty tissue surrounding each ovary was identified and removed. The ovary, along with its connecting ducts, was excised. Following this, the skin and muscle tissues were closed using non-absorbable 6-0 sutures. In the sham surgery group, the fat surrounding each ovary was simply retracted and replaced. Behavioral experiments were conducted on all animals one week post-ovariectomy (Heydarpour et al., 2013; Mirbaha et al., 2009).

Open-field test (OFT)

Mice underwent the open-field test before the FST and TST to reduce the potential impact of movement on the analysis of data related to despair-like behavior (David et al., 2003; Eckeli et al., 2000). The test requires a box measuring 40 cm in height, 60 cm in length, and 50 cm in width, with 12 squares removed from the floor. Every mouse is positioned in the field's left corner and given unrestricted mobility. During the 6-minute test, the number of squares the animal crossed with its paws was counted. Following each test, the equipment should be sanitized using a 10 percent ethanol solution (Shahsavarian et al., 2014).

Forced Swimming Test (FST)

The FST methods resembled those that Porsolt et al. first described in 1977. Every mouse was placed inside a glass cylinder that was 10 cm wide and 25 cm tall. The cylinder was filled with water up to 19 cm and maintained at a temperature of 25±1 °C, where they underwent 6 minutes of FST (David et al., 2003). The length of immobility was assessed in the final 4 minutes of the test, after an initial period of vigorous exercise of 2-3 minutes (Shahsavarian et al., 2014).

Tail Suspension Test (TST)

To assess immobility times in the TST, adhesive tape was used to suspend each mouse by its tail at a height of 50 cm, placed approximately 1 cm from the tip of the tail. The observer recorded the entire six-minute test period and calculated individual immobility times for each animal. Mice were considered immobile only when they hung down passively and were completely motionless (Saeedi Saravi et al., 2017).

Drugs

The medications used in this investigation included L-arginine, N(G)-nitro-L-arginine methyl ester (L-NAME) (Sigma, St Louis, MO, USA), and atorvastatin (Sobhan Company, Tehran, Iran). All drugs were given via intraperitoneal injection (diluted in physiological saline solution) at a constant volume of 10 mg per kilogram of body weight, except for the exception of atorvastatin (*administered* PO once daily *via gavage).*

Treatment

In the initial stage of the research, the immobility time was recorded in un-operated control, sham-operated, and untreated OVX groups, in which saline was injected as a vehicle to investigate the behavioral alteration of ovariectomy. Subsequently, the influence of atorvastatin on OVX mice was evaluated.

To achieve this, atorvastatin dosages of 0.01, 0.1, and 1 mg/kg were administered 1 hour before the behavioral experiments to evaluate the drug's impact on mice that had their ovaries removed.

To examine the hypothesis that the antidepressant-like effect of atorvastatin could be mediated by the nitric oxide pathway, the non-effective dose of L-NAME (10 mg/kg, i.p.) was co-administered with the lowest effective dose of atorvastatin (0.01 mg/kg) 45 minutes before FST and TST.

The effect of the NO precursor, L-arginine (750 mg/kg), was evaluated on the antidepressant-like activity of

a potent dose of atorvastatin (0.1 mg/kg) in the FST and TST. In this regard, L-arginine was administered 45 minutes before the administration of atorvastatin (Ghasemi et al., 2008; Heydarpour et al., 2013; Ludka et al., 2013).

Hippocampal nitrite concentration measurement

To assess how the nitric oxide pathway affects atorvastatin's ability to act as an antidepressant, nitrite levels in the hippocampus were measured (Boulton et al., 1994). Following the administration of mild anesthesia, animals were euthanized by decapitation, and their hippocampi were carefully removed on an ice-cold surface before being promptly frozen in liquid nitrogen. Subsequently, hippocampal tissue samples were obtained from the brain of each animal, following the completion of behavioral experiments (Amiri et al., 2015). After centrifuging the hippocampal homogenates at 40,000 g, the Griess reaction was performed (1% sulfanilamide, 2.5% H_3PO_4, and 0.1% N-(1-naphthyl)-ethylenediamine dihydrochloride). A standard plate reader was used to quantify the absorbance at 540 nm, and the sample was then incubated for 30 minutes at 37 °C. Fresh dilutions of nitrite standard solutions were utilized during each phase of the experiment. The overall levels of nitrite, which is the key metabolite of nitric oxide, were determined through linear regression analysis of the average values for each sample, with blank values subtracted.

Statistical analysis

In this experiment, the duration of immobility was assessed using Prism software and presented as mean±SEM. Group differences were analyzed using a one-way analysis of variance (ANOVA), followed by Tukey's post hoc test for further investigation. Statistical significance was determined by a P-value below 0.05 for all groups.

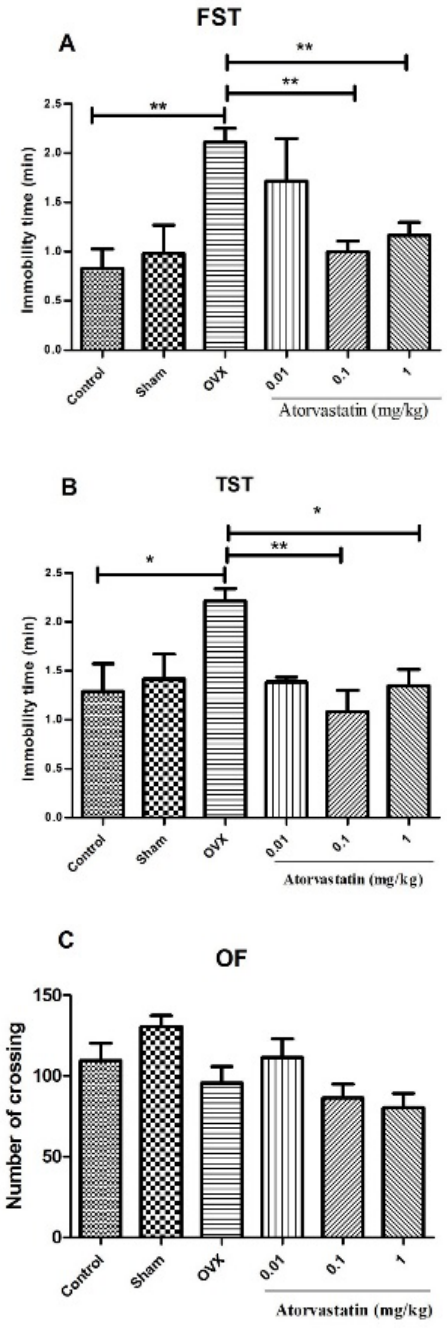

FIGURE 1. Effect of different doses of atorvastatin (0.01, 0.1 and 1 mg/kg) on the immobility time of mice in FST (A) and TST (B) and crosses made by OVX-mice in the OFT (C). Values are expressed as the mean ± S.E.M. Each group was composed of six mice. Data were analyzed using one-way ANOVA followed by Tukey's post-hoc test. *P < 0.05 and **P < 0.01 compared to the control groups.

Results

Effect of atorvastatin on behavioral tests in the OVX mice

In figures 1A and B, it was observed that ovariectomy led to a significant increase in immobility duration in both the FST and TST compared to the sham-operated group (P < 0.01, P < 0.05; Figure 1A). The number of crossings remained unchanged following ovariectomy in the OFT. Moreover, the antidepressant-like effects of atorvastatin (at doses of 0.01, 0.1, and 1 mg/kg; orally administered) were demonstrated when given one hour before the FST, TST, and OFT, as depicted in figures 1A, 1B, and 1C. A significantly higher level of antidepressant-like effects was noted after administering atorvastatin at dosages of 0.1 and 1 mg/kg compared to the other atorvastatin dosage (P < 0.01). Additionally, ator-

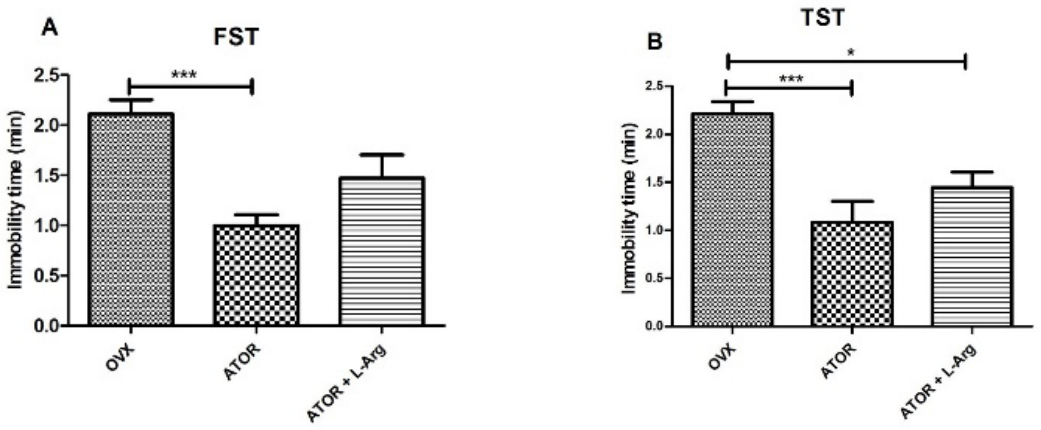

FIGURE 2. Effect of co-administration of L-Arginine (750 mg/kg) and atorvastatin (0. 1 mg/kg) on the immobility time of OVX mice in FST (A) and TST (B). Values are expressed as the mean ± S.E.M. Each group was composed of six mice. Data were analyzed using one-way ANOVA followed by Tukey's post-hoc test. *P < 0.05 and ***P < 0.001 compared to the corresponding control groups.

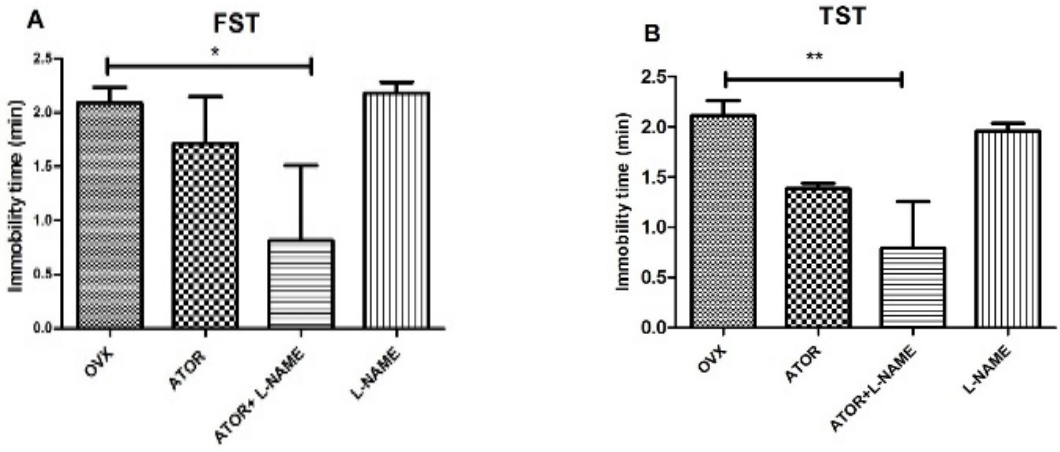

FIGURE 3. Effect of co-administration of L-NAME (10 mg/kg) and atorvastatin (0.01 mg/kg) on the immobility time of OVX mice in FST (A) and TST (B). Values are expressed as the mean ± S.E.M. Each group was composed of six mice. Data were analyzed using one-way ANOVA followed by Tukey's post-hoc test. *P < 0.05 and **P < 0.01 compared to the corresponding control groups.

vastatin does not have a noticeable impact on locomotor activity when comparing the number of square crossings with the sham-operated group in the OFT (P > 0.05; see Figure 1C). The results of the One-way ANOVA analysis revealed a notable impact of atorvastatin treatment on the FST ($F(5, 33) = 6.740$, $P<0.01$, Fig. 1A) and TST ($F(5, 38) = 3.934$, $P<0.05$, Fig. 1B). However, there were no statistically significant properties observed in the OFT ($F(5, 33) = 2.68$, $P>0.05$, Fig. 1C).

Effects of pretreatment with L-arginine on the antidepressant-like effects of atorvastatin in the OVX mice

As a precursor of nitric oxide synthesis, L-arginine was utilized to establish the impact of NO on the antidepressant-like properties similar to those of atorvastatin. The results revealed that administering L-arginine (750 mg/kg, i.p.) effectively counteracted the antidepressant-like response induced by atorvastatin (0.1 mg/kg, i.p.) in the FST and TST (P < 0.05; Fig. 2A and B).

Effects of L-NAME on the anti-immobility properties of atorvastatin

L-NAME, a non-selective NOS inhibitor, was used to explore the impact of nitric oxide on the antidepressant-like properties of atorvastatin. As shown in figures 3A and B, statistical analysis of one-way ANOVA

showed that co-administration of atorvastatin at a dose of 0.01 mg/kg (subeffective dose) and L-NAME (10 mg/kg) compared to either drug alone significantly reduced the amount of time the mice were immobile ($F(3, 20) = 3.275$, $P < 0.05$; Fig. 3A and $F(3, 16) = 7.904$, $P < 0.01$; Fig. 3B), in that order.

Examination of nitrite levels in the hippocampus

To explore the role of the NO system in the antidepressant properties of atorvastatin, the nitrite levels, an NO marker, in the hippocampal tissue of the experimental mice were quantified (Fig. 4). In contrast to the ovariectomy group, the levels of nitrite concentration in the hippocampus were notably reduced in the groups treated with atorvastatin at doses of 0.1 and 1 mg/kg ($F(5, 20) = 4.215$, $P<0.01$ and $P<0.05$, respectively; Fig 4A). The non-effective dose of atorvastatin, administered at 0.01 mg/kg, did not exhibit notable antidepressant-like properties in the FST and TST assays, and it also did not impact nitrite levels in OVX mice ($P > 0.05$). Ovariectomy was found to increase nitrite concentration in the hippocampus compared to the sham-operated group ($P < 0.05$). Concomitant co-administration of the lowest effective dose of atorvastatin (0. 1 mg/kg) with L-arginine resulted in a notable rise in hippocampal nitrite levels compared to atorvastatin (0.1 mg/kg)-treated mice ($P<0.05$; Fig. 4B). Co-administration of L-NAME with the sub-effective dose of atorvastatin (0.01mg/kg) did not significantly change the nitrite content of hippocampus ($P>0.05$; Fig 4C).

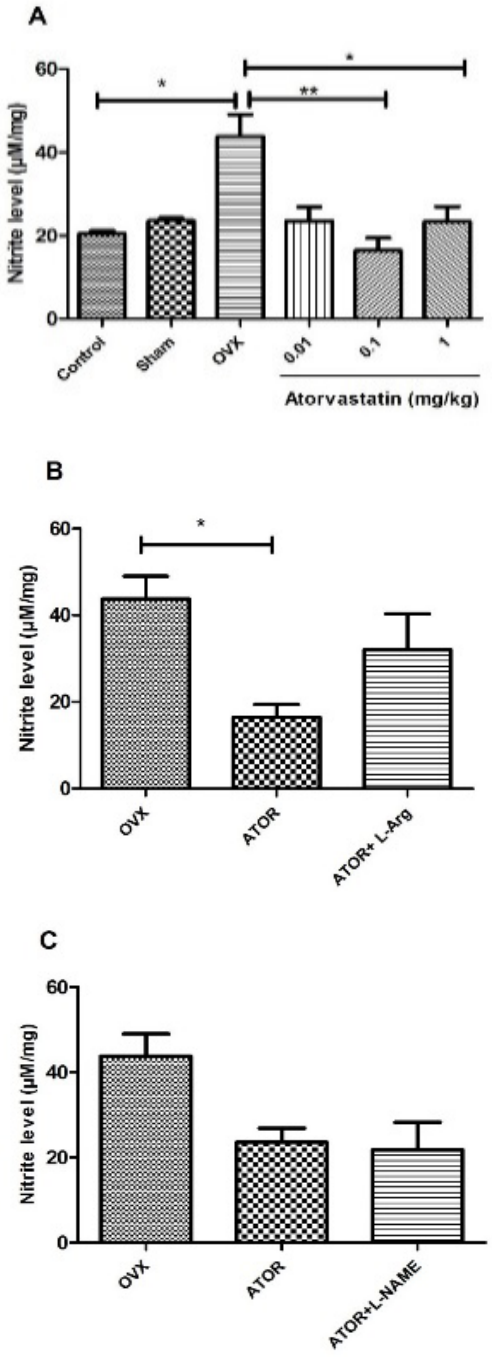

FIGURE 4. Effects of atorvastatin administration (A) and sub-effective and effective doses of atorvastatin with L-arginine and L-NAME, respectively, (B, C) on nitrite levels in the hippocampus. Values are expressed as the mean ± S.E.M. and were analyzed using one-way ANOVA followed by Tukey's post hoc test. Each group consisted of six mice. *$P < 0.05$ and ** $P < 0.01$ compared to the corresponding control groups.

Discussion

The current study examined the acute antidepressant-like effects of atorvastatin in the model of ovariectomy-induced depression and explored the potential role of the nitric oxide pathway in this process. In the animal model of FST and TST, the study showed that atorvastatin effectively alleviates the depressive symptoms triggered by ovariectomy. The maximum effect was achieved at a dose of 1 mg/kg, and this effect was dose-dependent. The study demonstrated an increase in immobility time after ovariectomy but no discernible change in locomotor behavior. To understand the mechanism of action of atorvastatin's antidepressant properties, it was shown that acute atorvastatin disrupts the NO/L-Arg signaling pathway. Specifically, the antidepressant-like properties of atorvastatin in FST and TST are attenuated when L-arginine, a precursor of nitric oxide synthase, is used, whereas L-NAME, a nonspecific nitric oxide synthase inhibitor, has the opposite effect. Following the behavioral tests, the study assessed the levels of nitrite, a stable byproduct of nitric oxide, in the

hippocampal homogenates of various groups to investigate the potential direct impact of atorvastatin on nitric oxide levels in the hippocampus. The results showed that atorvastatin had antidepressant-like properties and significantly reduced nitrite levels in the hippocampus at the same dosage. It is interesting to note that atorvastatin (0.01 mg/kg) did not change the amount of nitrite in the hippocampus and had no effect on the immobility behavior of ovariectomized mice during FST. These outcomes strongly suggest that NO is involved in the antidepressant properties of atorvastatin in this situation. Additionally, it was found that administering a non-effective dose of atorvastatin alongside a non-effective dose of the non-selective NOS inhibitor L-NAME to mice that had undergone ovariectomy produced a potent antidepressant impact. Overall, the study demonstrated that the iNOS inhibitor, L-NAME, significantly decreased the immobility time of atorvastatin-treated mice in FST and TST, indicating that atorvastatin exerts its anti-depressant-like effects at least partially through a pathway dependent on iNOS inhibition in OVX mice.

Previous research has observed potential antidepressant-like properties in male rodents treated with statins during tests such as the TST and FST (Lim et al., 2017; Shahsavarian et al., 2014). Our findings, which are in line with earlier research, show that ovariectomized mice exhibited longer periods of immobility without changing their locomotor activity during the TST and FST (Petit-Demouliere et al., 2005). Behaviors such as prolonged periods of immobility observed in the FST and TST are thought to be indicative of depression in humans (Saeedi Saravi et al., 2016). In depression models, statins—such as atorvastatin and lovastatin—have been demonstrated to have antidepressant-like effects. They can also amplify the antidepressant properties of other medications that cooperate with the serotonergic system and statins (David et al., 2003; Renshaw et al., 2009). Importantly, atorvastatin treatment prevented the prolongation of immobility time caused by LPS in both the TST and FST, indicating potential antidepressant properties (Taniguti et al., 2019). Possible ways in which statins may produce their antidepressant benefits could involve their ability to reduce inflammation and oxidative stress, impact monoamine neurotransmitters positively, promote neurotrophic effects, and lower lipid levels as a class of medication (Khler-Forsberg et al., 2020). In a clinical setting, it is recognized that statins can influence the serotonergic system by blocking the reuptake of synaptic neurotransmitters after they are released, and they also work in conjunction with SSRIs and SNRIs to enhance their effects (De Giorgi et al., 2023; Gutlapalli et al., 2022a). It is theorized that there is a link between cholesterol levels and serotonergic neurotransmission, which can indirectly impact the effectiveness of antidepressants (Gutlapalli et al., 2022b). In the last decade, research has demonstrated that atorvastatin has been found to have various effects, such as offering neuroprotective benefits in cases of brain injury-induced seizures and beta-amyloid toxicity (Lu et al., 2004; Piermartiri et al., 2010). In a study conducted by Young et al in 2003, it was suggested that atorvastatin may have beneficial effects on improving mood in individuals with depression. The study also discovered that atorvastatin has the potential to protect against the development of ischemia, thereby reducing the risk of mortality (Vandresen-Filho et al., 2013; Young-Xu et al., 2003). Additionally, research has demonstrated that atorvastatin has the potential to enhance cognitive, emotional, and impairment in rodent models of Alzheimer's disease and Parkinson's disease (Castro et al., 2013; Martins et al., 2015). It is hypothesized that atorvastatin's neuroprotective potential results from elevated nerve growth factor (NGF) levels in the hippocampus and striatum (Castro et al., 2013). Many research studies have shown that the therapeutic benefits of atorvastatin are due, at least in part, to its ability to reduce the inflammatory response. The positive influence of atorvastatin on alleviating Alzheimer's disease is confirmed, for example, by lower levels of hippocampal IL-1β, IL-6, and tumor necrosis factor alpha (Zhang et al., 2013). Recent research has indicated that elevated tryptophan levels and heightened tPA activity, which are critical for the conversion of pro-BDNF to BDNF, are possible mechanisms for the antidepressant properties of atorvastatin (Pang et al., 2004; Seidaha et al., 1996).

Following the discovery of antidepressant-like properties of NOS inhibitors by Harkin and colleagues, converging lines of evidence emerged pointing to the role of NO and NOS in the pathophysiology of depression (Harkin et al., 1999). It has been demonstrated that atorvastatin has an impact on the nitric oxide signaling pathway. When pioglitazone and L-NAME are given together with an ineffective dose of atorvastatin, it has been observed to decrease immobility time in FST and

TST. On the other hand, the results were reversed when L-Arginine and GW9662 were given in conjunction with atorvastatin (Shahsavarian et al., 2014). Clinical research has suggested that mice given statins show reduced levels of oxidative stress and nitric oxide, potentially leading to enhanced protection against ischemic brain injury in animal models (Asahi et al., 2005). Furthermore, studies in clinical settings have shown that the antidepressant-like impact of atorvastatin is controlled by its ability to inhibit NO-cGMP synthase and NMDA receptors, as well as reducing NO levels in the hippocampus (Piermartiri and Vandresen-Filho 2009). Suzuki and colleagues proposed in 2001 that increased levels of nitrate in the bloodstream can contribute to depressive symptoms by triggering inflammatory reactions that can be destructive for the central nervous system. This damage may result in impaired neuronal function and the onset of severe mental illnesses (Suzuki et al., 2001). It is worth mentioning that atorvastatin has the potential to impact the hippocampus, which is identified as a key component of the limbic system responsible for regulating emotional disorders like depression and anxiety (Saeedi Saravi et al., 2017). In accordance with our findings, a number of other authors have demonstrated that decreasing the level of nitric oxide or blocking its production in the brain may lead to antidepressant benefits (Joca and Guimares 2006). Hence, the intrinsic amount of nitric oxide (NO) within the hippocampus is crucial in the development of major depressive disorder (MDD) (Maes 1999). This phenomenon could elucidate the decrease in immobility duration observed in the FST after L-NAME treatment, indicating its antidepressant properties (Ghasemi et al., 2008). A study by Montezuma et al. (2012) illustrated that inhibiting iNOS or its knockdown can trigger antidepressant-like responses. This suggests that the production of NO mediated by iNOS is involved in behavioral responses to stress and the neural processes associated with depression (Montezuma et al.).

These results align with the data indicating that persistent psychological stress factors increase NOS activity, as well as iNOS protein and mRNA levels in brain cells, and elevate nitrite levels in rodents' bloodstreams. These impacts can be prevented by using a specific iNOS inhibitor (Olivenza et al., 2000; Peng et al., 2012). Taken together, these findings indicate that NOS inhibitors may have a promising therapeutic application in conditions related to stress (Lisboa et al., 2015). The presence of nNOS in various mammalian brain regions coincides with the location of receptors for gonadal hormones like estrogen receptors (ERalpha and ERbeta), progesterone receptors (PR), and androgen receptors (AR) (Panzica et al., 2006). The disparity in depression-like behaviors between sexes is not solely attributed to estrogen; nitric oxide (NO) in the hippocampus also play a key role in mediating this sex difference and directly influencing behaviors. Studies have verified the critical involvement of hippocampal NO in the behavioral impacts of estrogen (Hu et al., 2012). As demonstrated in studies, the administration of estradiol has been discovered to notably reduce NO levels in the hippocampus, leading to antidepressant-like properties in ovariectomized mice during the FST (Heydarpour et al., 2013). Indeed, in line with its dual role in the peripheral system, nitric oxide shows differential effects in depression, potentially influenced by its intricate interaction with regulators and signaling pathways (Heydarpour et al., 2013; Joca and Guimares 2006).

In our experiment, sex hormone depletion (ovariectomy) resulted in an increase in NO concentrations in the hippocampus, which was linked to behaviors resembling depression in the FST. Nonetheless, nonselective NOS inhibitors exhibited beneficial effects in alleviating depression, indicating that regulating NO levels could offer a potential therapeutic strategy for depression. Consistent with our findings, Joca and Guimarães supported the depressive role of NO by illustrating that in the rat hippocampus, blocking nNOS resulted in antidepressant-like properties (Joca and Guimares 2006). Consistent with prior research, the current study demonstrated heightened nitrite levels in the hippocampus of OVX mice, indicating neuro-inflammation in this model after estrogen depletion, potentially leading to depression (Menze et al., 2021). Therefore, suppressing neuroinflammation might be the fundamental process responsible for estrogen's protective effects on the brain. Our study provides insight into the mechanism by which atorvastatin, an anti-inflammatory agent, exhibits antidepressant-like properties, at least in part via NO in the FST and TST of the OVX mouse.

Less than two-thirds of people with depression find antidepressant *medications* effective. Therefore, there is a need to develop new therapeutic strategies for major depression with *improved* efficacy (Rostamian et al.,

2019). It is worth noting that the prevalence of major depression and anxiety in women is approximately twice that of men (Hu et al., 2012). Novel anti-inflammatory drugs, such as statins, which regulate NOS function in the brain, could be very effective in treating depression. Our findings suggest that atorvastatin's antidepressant effect in ovariectomized mice may be related to the nitric system. This could provide a foundation for developing new antidepressants with increased efficacy for women experiencing perimenopausal affective disorders.

Conclusions

In summary, our research provides insight into the process by which atorvastatin, a medication used to lower cholesterol levels, demonstrates a significant antidepressant effect in OVX mice in the FST and TST, possibly by blocking NOS activity. Our findings further support the role of NO and inflammation in the onset of depression. Further investigation is necessary to elucidate the impact of anti-inflammatory drugs and NOS inhibitors on depressive symptoms. These studies would offer valuable insights into the intricate network of *signaling pathways* involved in depression associated with low estrogen levels in women.

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Conflict of interest declaration

There is no conflict of interest.

Funding

None

Ethics approval statement

All procedures and manipulations were carried out in accordance with the guidelines of the Ethics Committee of Shahid Sadoughi University of Medical Sciences (IR.SSU.MEDICINE.REC.1396.90) and in accordance with the NIH Guide for the Care and Use of Laboratory Animals (publication no. 85–23, revised 1985).

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