Archives of Razi Institute, Vol. 76, No. 5 (2021) 1407-1417

DOI: 10.22092/ari.2020.352221.1553

Original Article

Anti-depressant Effect of Betaine Mediates via Nitrergic and Serotoninergic Systems in Ovariectomized Mice

Haramipour, P[1], Asghari, A[2], Hassanpour, Sh[3 *], Jahandideh, A[2]

1. Faculty of Veterinary Medicine, Science and Research Branch, Islamic Azad University, Tehran, Iran
2. Department of Clinical Sciences, Faculty of Veterinary Medicine, Science and Research Branch, Islamic Azad University, Tehran, Iran
3. Division of Physiology, Department of Basic Sciences, Faculty of Veterinary Medicine, Science and Research Branch, Islamic Azad University, Tehran, Iran

Received 19 October 2020; Accepted 21 November 2020
Corresponding Author: s.hassanpour@srbiau.ac.ir

Abstract

This study aimed to determine the anti-depressant effect of betaine (BT) in ovariectomized mice and its possible interaction with nitrergic and serotoninergic systems. In experiment 1, the mice were divided into control and sham groups, ovariectomy (OVX), OVX+BT (12.5mg/kg), OVX+BT (25 mg/kg), and OVX+BT (50mg/kg) groups. In experiment 2, the mice were assigned into control and sham, OVX, OVX+BT (50mg/kg), OVX+L-NAME (10 mg/kg), as well as OVX+injection of the BT and L-NAME. Experiments 3-5 were similar to experiment 2, except for L-Arginine (50 mg/kg), Fluoxetine (5 mg/kg), and Cyproheptadine (4 mg/kg) that were injected instead of the L-NAME. Subsequently, forced swimming test (FST), tail suspension test (TST), and open field test (OFT) were performed in this study. Moreover, this study determined serum Malondialdehyde (MDA), Superoxide dismutase (SOD), Glutathione peroxidase (GPx), and total antioxidant status levels. According to the findings, OVX increased immobility time, compared to the control group ($P<0.05$). In addition, BT (50mg/kg) decreased depression-induced immobility time, compared to the OVX group ($P<0.05$). The co-injection of the BT+L-NAME decreased depression-induced immobility time in TST and FST, followed by an increase in the number of crossing in OFT($P<0.05$).Moreover, the co-injection of the BT+L-Arginine significantly diminished the antidepressant activity of BT on immobility time and decreased positive effect of BT on the number of crossing ($P<0.05$). The co-injection of the BT+Fluoxetine significantly amplified the antidepressant activity of BT on immobility time and number of crossing ($P<0.05$). Furthermore, the co-injection of the BT+Cyproheptadine decreased antidepressant activity of BT on immobility time and number of crossing ($P<0.05$). The BT (25 and 50mg/kg) reduced the MDA; however, it elevated SOD and GPx levels in OVX mice ($P<0.05$). It seems that antidepressant activity of BT mediates via nitrergic and serotoninergic systems in OVX mice.

Keywords: Anti-depressant, Betaine, Serotoninergic, Nitrergic, Ovariectomy, Mice

1. Introduction

Depression is one of the main mental disorders which is categorized by impairment in mood, interest, or pleasure and can eventually lead to suicide (1). Major depressive disorder (MDD) is a complex which impresses brain physiological function and alters emotional and cognitive processes. The incidence and prevalence of MDD is higher in women than men because of hormonal fluctuations and increases in the menopausal stage (2). Ovarian hormones have a prominent role in emotional perception, mood regulation, response to stress, and cognition. Alterations in the estradiol milieu during the menstrual cycle, parturition, and menopause increase depression

risks and mood disruption in women (3). Bilateral ovariectomy in mice leads to depressive-like behaviors (4), and estradiol therapy showed antidepressant-like effects in ovariectomized (OVX) mice (5). Although the direct mechanism for the effects of estradiol in mood and depression is not fully elicited, it acts by the modulation of the dopaminergic, serotonergic, and nitric oxide systems (5, 6).

Nitric oxide (NO) is a free radical gas, produced from L-Arginine as a result of NO synthase (NOS) and has several physiological roles in the brain (7). The NO has a crucial role in the pathogenesis of depression by its modulatory effects on serotonin, dopamine, and norepinephrine (8). The fluctuations of the NO concentrations in the hippocampus during the ovarian cycle may suggest that the nitrergic system has a mediatory role in the effects of estrogen (9). The hippocampus plays a key role in MDD (10). Estrogen deficiency leads to increased hippocampus NO levels, and the administration of L-N^G-Nitro arginine methyl ester (L-NAME, nitric oxide synthesis inhibitor) in the rat hippocampus decreases NO levels and exerts antidepressant-like effects (11). Based on the evidence, nitrergic neurons have synapses with serotoninergic neurons through which NO modulates serotonin release, reuptakes, and functions the same as its extracellular levels. Serotonin is the main neurotransmitter for the regulation of mood, and its deficiency leads to depression (12).

There are several antidepressant medications for the pathogenesis of MDD, such as selective serotonin reuptake inhibitors and tricyclic anti-depressants which affect the monoaminergic system. However, sedation, seizures, and sexual dysfunction are major side effects of these antidepressants. Therefore, there is growing interest in new antidepressant agents from natural and bioactive products. Betaine (BT) (glycine betaine or trimethylglycine) is a natural product primarily isolated from the *Beta vulgaris* plant, other plants, and microorganisms.

Moreover, it can inhibit nuclear factor-κB and cyclooxygenase-2, inducible nitric oxide synthase (iNOS), and tumor necrosis factor (13). Betaine has positive effects against biological stresses, and its levels decreased in schizophrenia patients (14). Furthermore, it has positive effects against memory impairmentin mice (13). Immobility time in forced swimming test (FST) resembles a state of despair. Tail suspension test (TST) and FST are useful methods for depression-like behaviors in rodents. These tests reflect depressive behaviors in humans. The BT plays a major role in improving individuals' mood with mild-to-moderate depression (15). In the hippocampus and hypothalamus, BT alters serotonin levels in FST (1), and its injection reduced the immobility time in rats (16). Despite its antidepressant effects, there is no information about its antidepressant activity with sex hormone deficiency-related depression. Therefore, this study aimed to determine the antidepressant effects of BT in OVX mice and its possible interaction with the nitrergic and serotoninergic systems.

2. Materials and Methods

2.1. Animals

A total of 240 adult female NMRI mice (weighing 28-30 g and aging8-10 weeks old) were supplied from the Pasteur Institute, Tehran, Iran. The animals were kept at the physiology laboratory of Science and Research Branch, Islamic Azad University, Tehran, Iran, according to the Guide for the Care and Use of Laboratory Animals by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

2.2. Drugs

The BT, L-NAME (nitric oxide inhibitor, 10mg/kg), L-Arginine (nitric oxide processor), Cyproheptadine (serotonergic receptor antagonist), and Fluoxetine (selective serotonin reuptake inhibitor) were purchased from Sigma Aldrich (St, USA).

2.3. Experimental OVX

Following anesthesia by intraperitoneally (IP) injection of ketamine hydrochloride (50 mg/kg) and xylazine hydrochloride (5 mg/kg) (Alfasan, Woerden, Holland), the lumbar dorsum was shaved, and the exposed skin was scrubbed by a sterile saline wipe and

10% povidone-iodine. A 1-2-cm incision was made on the midline of the lumbar vertebral line. One cm to each flank, parovarian fatty tissue was taken out, the ovary and associated oviduct were removed, and skin incision was sutured (4-0 non-absorbable). In the sham group, the parovarian fatty tissues and ovaries were just retracted and replaced (17-20). All behavioral tests were performed 10 days after recovery (5).

2.4. 17β-Estradiol Assay

Blood samples were taken from each mouse via cardiac puncture for 17β-estradiol (E_2) using the direct and competitive chemiluminescence immunoassay detection by the LIASION Estradiol (310400) kit.

2.5. Experimental Procedure

Following that, the OVX mice were randomly allocated into five experimental groups (48mice in each experiment, and mice in each group). Experiment 1 included a control group (A) without surgery that was injected with saline (10 ml/kg) one hour before the test, a sham group (B) which had no OVX and was injected with saline (10 ml/kg) one hour before the test, OVX (C) mice IP injected with saline (10 mL/kg) one hour before the test, OVX (D) mice injected with BT (12.5 mg/kg) one hour before the test, OVX(E) mice injected with BT (25 mg/kg) one hour before the test, and OVX (F) mice IP injected with BT (12.5 mg/kg) one hour before the test.

Experiment 2 included a control group (A) without surgery was injected with saline (10 mL /kg) one hour prior to the test, a sham group (B) with no OVX that was injected with saline (10 ml/kg) one hour before the test, an OVX group (C) that was IP injected with saline (10 ml/kg) one hour before the test, an OVX group (D) that was injected with BT (50 mg/kg) one hour before the test, an OVX group (E) that was injected with L-NAME (10mg/kg) one hour before the test, and an OVX group (F) that was injected with BT (50 mg/kg) one hour prior to test. Following that, 15 min after the final injection, the animals were IP injected with L-NAME (10 mg/kg), and 45 min later, the tests were conducted.

Experiment 3 consisted of a control group (A) without surgery that was injected with saline (10 ml/kg) one hour before the test, a sham group(B) with no OVX that was injected with saline (10 mL /kg) one hour before the test, an OVX group (C) that was injected with saline (10 mL /kg) one hour prior to test, an OVX group (D) that was injected with BT (50 mg/kg) one hour before the test, an OVX group(E) that was injected with L-Arginine (50mg/kg) one hour before the test, and an OVX group (F) that was injected with BT (50 mg/kg) one hour before the test. Afterward, 15 min later, they were IP injected with L-Arginine (50mg/kg), and 45 min later, the tests were conducted.

Experiment 4 included a control group (A) without surgery that was injected with saline (10 mL /kg) one hour before the test, a sham group (B) with no OVX that was injected with saline (10 ml/kg) one hour prior to the test, an OVX group (C) that was IP injected with saline (10 ml/kg) one hour before the test, an OVX group (D) that was injected with BT (50 mg/kg) one hour before the test, an OVX group (E) that was injected with fluoxetine (5mg/kg) one hour before the test, and an OVX group (F) that was injected with BT (50 mg/kg) one hour before the test. It should be mentioned that 15 min after the final injection, the animals were IP injected with fluoxetine (5mg/kg), and 45 min later, the tests were conducted.

Experiment 5 consisted of a control group (A) without surgery that was injected with saline (10 ml/kg) one hour before the test, a sham group (B) with no OVX that was injected with saline (10 mL /kg) one hour before the test, an OVX group(C) that was injected with saline (10 ml/kg) one hour before the test, an OVX group (D) that was injected with BT (50 mg/kg) one hour prior to the test, an OVX group (E) that was injected with cyproheptadine (4mg/kg) one hour before the test, and an OVX group (F) that was injected with BT (50 mg/kg) one hour before the test. It is worth mentioning that 15 min after the final injection, the animals were IP injected with cyproheptadine (4mg/kg), and 45 min later, the FST, TST, and OFT were conducted. At the end of the

study, serum MDA, SOD, GPx, and TAS levels were determined.

2.6. Forced Swimming Test

The FST was performed using the described protocol in mice (21). The animal was plunged into a glass cylinder containing 25±1°C water for 15 min (pre-test session). The test was repeated 24 h later for 6 min (test session). When the mouse ceased struggling and remained floating motionless in the water, the immobility time was recorded as the total period of immobility during the last 4 min of the 6 min.

2.7. Tail Suspension Test

It is known as a common antidepressant-like activity in mice (22). According to Steru, Chermat (23), briefly, the mice were kept away from any objects nearby and then suspended above the floor from the extremity of the tail. Immobility time was recorded for 6min.

2.8. Open Field Test

The OFT was used to determine the possible effects of BT on locomotor and exploratory activities. The open field was performed using a 45×45×30 cm^3 poly wood cage. The floor of the open field cage was divided by masking tape markers into 3×3 cm^2 squares. The mice were placed individually and observed for 6 min for the number of segments crossed with four paws (24).

2.9. Antioxidant Activity

At the end of the tests, blood samples were collected via cardiac puncture and serum Malondialdehyde (MDA), Superoxide dismutase (SOD), Glutathione peroxidase (GPx), and TAS that were determined using Zell Bio GmbH assay (Germany).

2.10. Statistical Analysis

The obtained data were analyzed by one-way analysis of variance (ANOVA). For treatments found to have an effect according to the ANOVA, mean values were compared with Tukey's test. The data were presented as the mean±SEM, anda p-value less than 0.05was considered statistically significant.

3. Results

The results of the anti-depressant and antioxidant effects of BT in OVX mice are presented in Figures 1-5 and table 1. A serum E_2 level of lower than 20±2 pg/ml was used for the accuracy of the OVX (5). As can be observed in figure 1, no significant differences were found between the sham and control groups in terms of immobility time in the FST and TST (P>0.05). The OVX significantly increased immobility time in FST and TST, compared to the control group (P<0.05). The BT (12.5 mg/kg) had no effect on immobility time (P>0.05), while doses of 25 and 50 mg/kg decreased depression-induced immobility time, compared to the OVX group (P<0.05) in a dose-dependent manner.

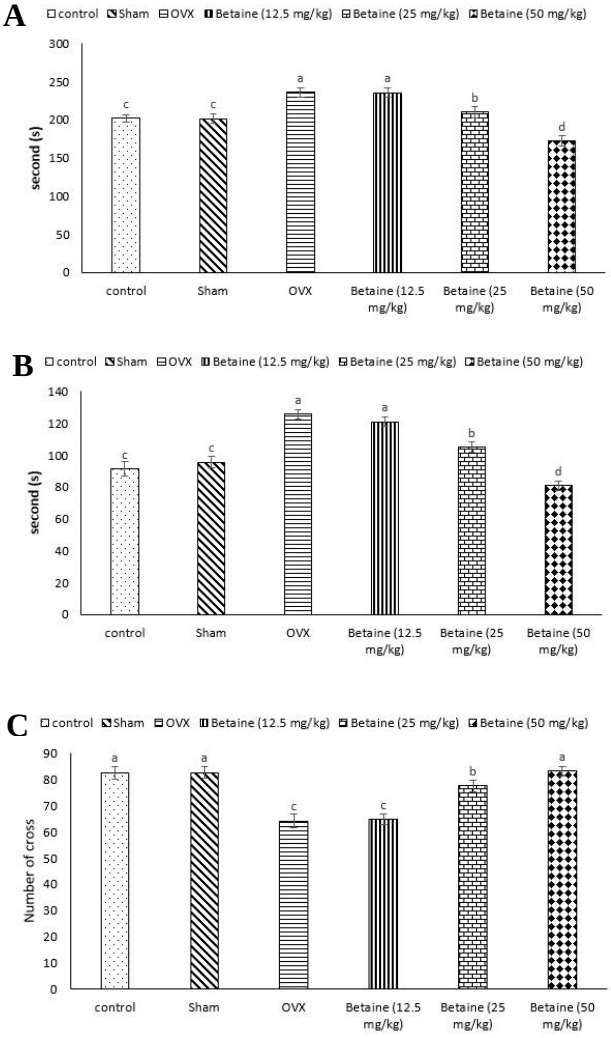

Figure 1. Effects of Betaine (12.5, 25, and 50 mg/kg) on FST **(A)**, TST, **(B)**, and OFT **(C)** in ovariectomized mice. Different letters (a-c) indicate significant differences amongtreatments (P<0.05)
TST: tail suspension test, FST: forced swimming test, OFT: open field test

There was no significant difference between sham and control groups regarding the number of crossings in the OFT (P>0.05). The OVX significantly decreased the number of crossing in the OFT, compared to the control group (P<0.05). The BT had no effect on the OFT (P>0.05) at the 12.5 mg/kg level, while 25 and 50 mg/kg of the BT significantly increased the number of crossings in the OFT, compared to the OVX mice (P<0.05).

As shown in figure 2, no significant difference was observed between the control and sham groups in terms of immobility time in FST and TST (P>0.05). The OVX significantly increased immobility time in FST and TST, compared to the control group (P<0.05). The BT (50 mg/kg) significantly reduced depression-induced immobility time, compared to the OVX group (P<0.05). The L-NAME (10 mg/kg) had no effect on immobility time, compared to the OVX mice (P>0.05). The co-injection of the BT (50 mg/kg)+L-NAME (10 mg/kg) significantly decreased depression-induced immobility time, compared to the control group (P<0.05). The OVX significantly diminished the number of crossings in the OFT, compared to the control mice (P<0.05). The BT (50 mg/kg) significantly augmented the number of crossings in the OFT, compared to the OVX group (P<0.05). The L-NAME (10 mg/kg) had no effect on the OFT, compared to the OVX group (P>0.05). The co-injection of the BT (50 mg/kg)+L-NAME (10 mg/kg) significantly amplified the number of crossings, compared to the OVX mice (P<0.05).

According to the results, immobility time significantly was increased in the OVX group, compared to the control group (P<0.05). The BT (50 mg/kg) significantly lessened immobility time, compared to the OVX mice (P<0.05). The L-Arginine (50 mg/kg) had no effect on the immobility time, compared to the OVX mice (P>0.05). The co-injection of the BT (50 mg/kg)+L-Arginine (50 mg/kg) significantly decreased the antidepressant activity of the BT on immobility time, compared to the OVX group (P<0.05). The OVX significantly reduced the number of crossings, compared to the OVX mice (P<0.05). Furthermore, 50 mg/kg of the BT significantly increased the number of crossings, compared to the OVX mice (P<0.05). The co-injection of the BT (50 mg/kg)+L-Arginine (50 mg/kg) decreased the positive effect of the BT on the number of crossings, compared to the OVX group (P<0.05). It seems that the antidepressant activity of the BT is mediated via the nitrergic system in OVX mice (Figure 3).

Based on figure 4, the OVX significantly increased immobility time in the FST and TST, compared to the control mice (P<0.05). The BT (50 mg/kg) significantly reduced depression-induced immobility time, compared to the OVX group (P<0.05). Fluoxetine (5 mg/kg) had no effect on immobility time, compared to the OVX mice (P>0.05). The co-injection of the BT (50 mg/kg)+fluoxetine (5 mg/kg) significantly amplified the antidepressant activity of the BT on immobility time, compared to the OVX group (P<0.05). The OVX significantly reduced the number of crossings, compared to the OVX mice (P<0.05).

A total of 50 mg/kg of the BT significantly increased the number of crossings, compared to the OVX group (P<0.05). The co-injection of the BT (50 mg/kg)+fluoxetine (5 mg/kg) increased the positive effect of the BT on the number of crossings, compared to the OVX mice (P<0.05).

As shown in figure 5, OVX significantly increased immobility time in the FST and TST, compared to the control group (P<0.05). The BT (50 mg/kg) significantly reduced depression-induced immobility time, compared to the OVX mice (P<0.05). The cyproheptadine (4 mg/kg) had no effect on immobility time, compared to the OVX group (P>0.05). The co-injection of the BT (50 mg/kg)+cyproheptadine (4 mg/kg) significantly decreased the antidepressant activity of the BT on immobility time, compared to the OVX group (P<0.05). The OVX significantly reduced the number of crossings, compared to the

OVX mice (P<0.05). The BT (50 mg/kg) significantly increased the number of crossings, compared to the OVX group (P<0.05). The co-injection of the BT (50 mg/kg)+cyproheptadine (4 mg/kg) decreased the positive effect of the BT on the number of crossings, compared to the OVX mice (P<0.05). This may suggest that the antidepressant activity of the BT is mediated via the serotoninergic system in OVX mice.

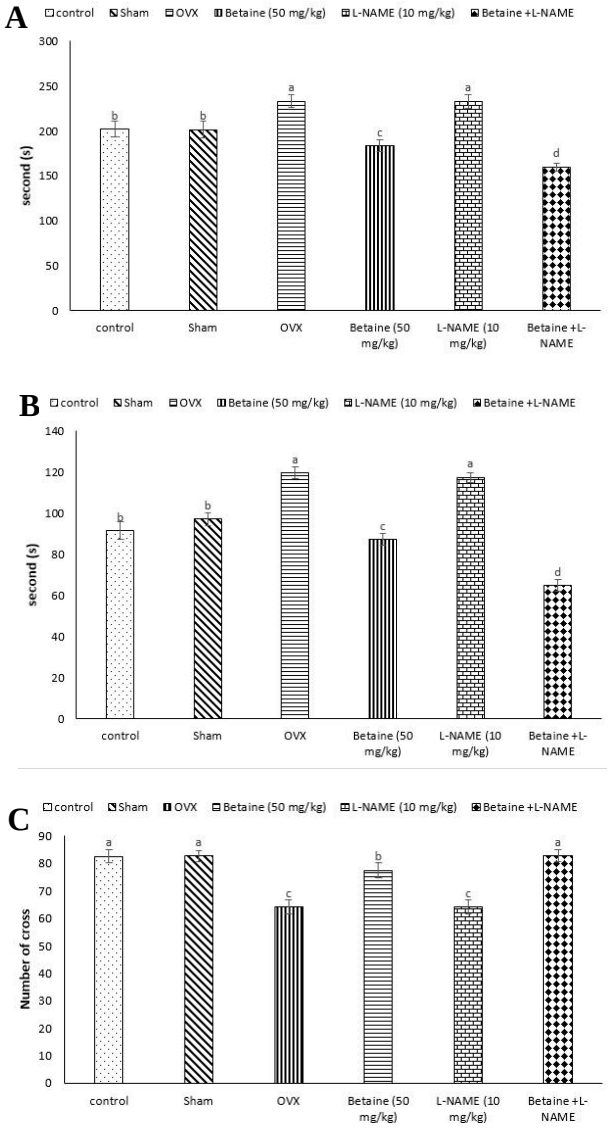

Figure 2. Effects of Betaine (50 mg/kg), L-NAME (10 mg/kg), and their co-injection on FST **(A)**, TST **(B)**, and OFT **(C)** in ovariectomized mice. Different letters (a-c) indicate significant differences amongtreatments (P<0.05). L-NAME: L-N^G-Nitro Arginine methyl ester (L-NAME), TST: tail suspension test, FST: forced swimming test, OFT: open field test

As can be observed in table 1, OVX significantly increased the MDA levels, compared to the control group (P<0.05). The BT significantly reduced the MDA levels, compared to the OVX mice (P<0.05). The SOD and GPx levels significantly diminished following OVX (P<0.05). The BT (25 and 50 mg/kg) significantly elevated the SOD and GPx levels, compared to the OVX mice (P<0.05). However, there was no significant difference in TAS (P>0.05).

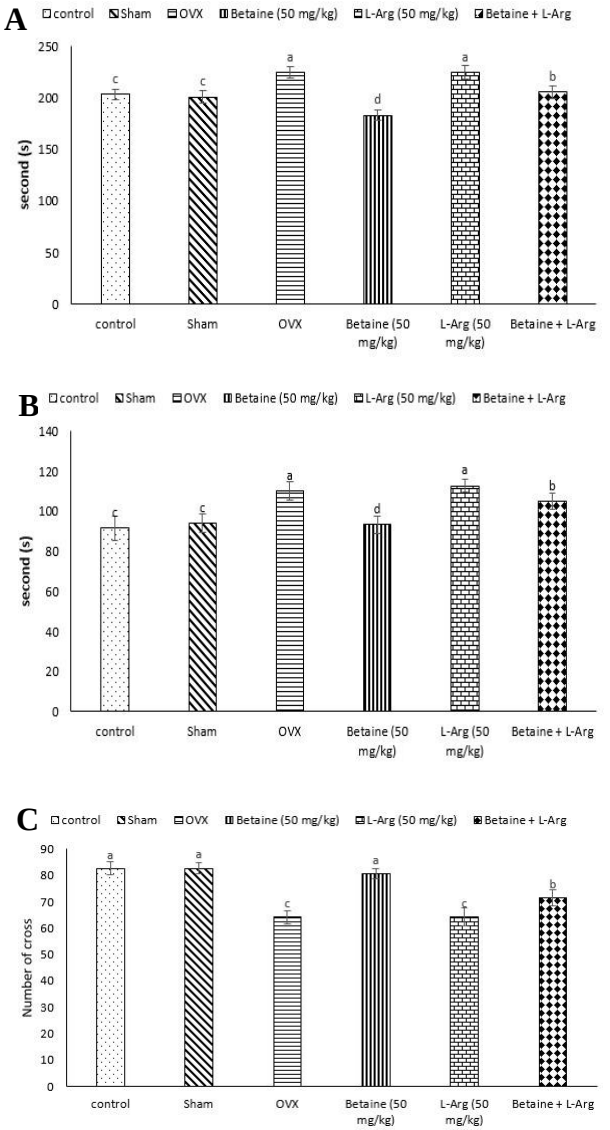

Figure 3. Effects of Betaine (50 mg/kg), L-Arg (50 mg/kg), and their co-injection on FST **(A)**, TST **(B)**, and OFT **(C)** in ovariectomized mice. Different letters (a-d) indicate significant differences amongtreatments (P<0.05). L-Arg: L-Arginine,TST: tail suspension test, FST: forced swimming test, OFT: open field test

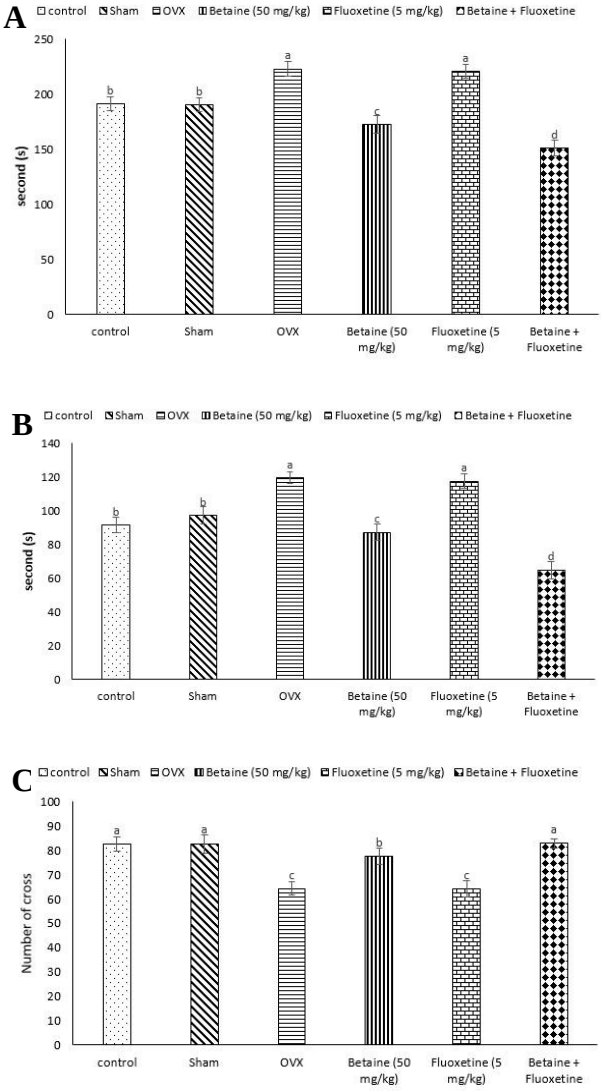

Figure 4. Effects of Betaine (50 mg/kg), Fluoxetine (5 mg/kg), and their co-injection on FST **(A)**, TST **(B)**, and OFT **(C)** in ovariectomized mice. Different letters (a-d) indicate significant differences amongtreatments (P<0.05). TST: tail suspension test, FST: forced swimming test, OFT: open field test

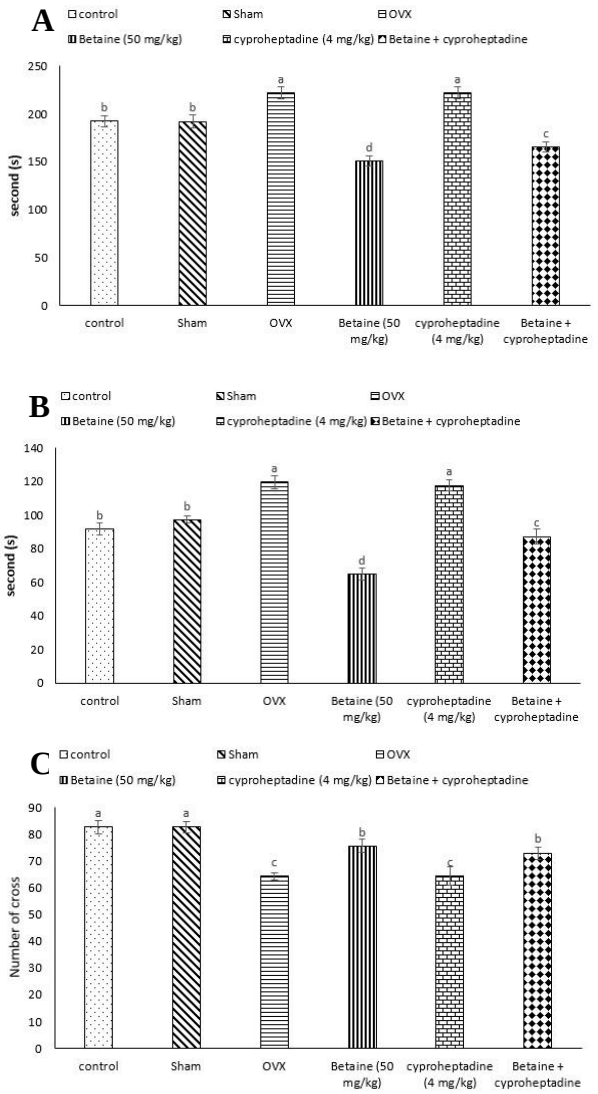

Figure 5. Effects of Betaine (50 mg/kg), Cyproheptadine (4 mg/kg), and their co-injection on FST **(A)**, TST **(B)**, and OFT **(C)** in ovariectomized mice. Different letters (a-d) indicate significant differences amongtreatments (P<0.05). TST: tail suspension test, FST: forced swimming test, OFT: open field test

Table 1. Effect of different levels of Betaine on serum antioxidant enzyme values in ovariectomized mice

Group	MDA(nmol/ml)	SOD (IU/ml)	GPx (IU/ml)	TAS (nmol/ml)
Control	5.86±0.24 ^{c}	65. 84±2.13 ^{a}	6.24±0.26 ^{a}	1.85±0.02
Sham	6.01±0.32 ^{c}	53.50±2.11 ^{a}	6.35±0.16 ^{a}	1.84±0.01
OVX	12.31±0.11 ^{a}	17.66±1.11 ^{d}	2.23±0.11 ^{d}	1.80±0.04
Betaine (12.5 mg/kg)	11.58±0.25 ^{a}	17.65±1.16 ^{d}	2.17±0.23 ^{d}	1.82±0.02
Betaine (25 mg/kg)	8.46±0.13 ^{b}	31.66±1.21 ^{c}	3.42±0.16 ^{c}	1.83±0.03
Betaine (50 mg/kg)	6.56±0.24 ^{c}	51.53±1.18 ^{b}	4.54±0.11 ^{b}	1.81±0.01

OVX: ovariectomy, MDA: malondialdehyde, SOD: superoxide dismutase, GPx: glutathione peroxidase, TAS: total antioxidant status. Different letters (a-d) indicate significant differences amongtreatments (P<0.05), (n=10 per group).

4. Discussion

The effects of the gonadal E_2 on emotions and mood, depression, and cognitive behavior are complex and differ during the reproductive life stage. In women with MDD vulnerability, E_2 may support the healthy functioning of these areas in the brain, whereas low E_2 levels increase the risk for depressive episodes (3). Rodents are ideal models for depressive-like states, and strain, age at OVX, as well as the time of behavioral test, following OVX, influence the results (5). In our study, the serum E_2 levels on adult female NMRI mice below 20±2 pg/ml were taken as OVX which were similar to previous reports (1, 5). It has been reported that E_2 decreases the latency to the beginning of antidepressants in TST and FST. Even though the direct mechanism for antidepressant-like actions of E_2 is not well-understood, its effects are mediated via involving E_2 receptors (ERα and ERβ), serotonergic, dopaminergic, and noradrenergic receptors in the amygdala and hippocampus (8). Moreover, E_2 modulates neural NOS (nNOS) expression by activating ERβ in hippocampal neurons. Additionally, E_2 modulates serotonin receptor mRNA levels in a genetic model of depression in mice (5). Finally, serotonin suppresses nNOS expression in the hippocampus in anxiety-related behavior (25). Nitric oxide has a key role in the pathogenesis of MDD, and its levels increase in the hippocampus of these patients (8). In this regard, Heydarpour, Salehi-Sadaghiani (5) reported that the non-selective NOS inhibitor (L-NAME, 30 mg/kg, IP) decreased immobility time in the OVX mice. Furthermore, L-NAME (10 mg/kg), 15 minutes after a sub-effective dose of the E_2 (1 μg/kg), had a positive antidepressant-like effect on the OVX mice which indicates the involvement of hippocampal NO signaling in the antidepressant activity of the E_2.However, based on the limitations of the current study, NO levels following OVX could not be determined in this study.

Our present findings suggest that OVX increased immobility time, and BT (25 and 50 mg/kg) decreased depression-induced immobility time in FST and TST. A sub-effective dose of L-NAME (10 mg/kg) had no effect on immobility time; however, the co-injection of the BT+L-NAME decreased depression-induced immobility time in OVX mice. In addition, a sub-effective dose of the NO processor (L-Arginine, 50 mg/kg) had no effect on immobility time. On the other hand, the co-injection of the BT and L-Arginine significantly decreased the antidepressant activity of the BT on immobility time in the OVX mice.

A sub-effective dose of the selective serotonin reuptake inhibitor (fluoxetine, 5 mg/kg) had no effect on immobility time in the TST and FST, while the co-injection of the BT and fluoxetine significantly amplified the antidepressant activity of the BT. In contrast, a sub-effective dose of the serotonergic receptor antagonist (cyproheptadine, 4 mg/kg) had no effect on immobility time; nonetheless, the co-injection of the BT and cyproheptadine decreased the antidepressant activity of the BT in the OVX mice.

In a previous report, Kim, Lee (1) reported that BT (30 and 100 mg/kg, IP) significantly decreased immobility time in FST, which was in line with the findings of the present study. Additionally, they reported that the effect of the BT (30 mg/kg) was quite close tothat of fluoxetine (10 mg/kg, as control) (1).

The BT played a major role in improving individuals suffering from mild-to-moderate depression (15) and the injection of 30 and 100 mg/kg BT decreased immobility time in FST in rats (16). Most antidepressants act by increasing serotonin and norepinephrine levels in the hippocampus, limbic, thalamic, and prefrontal cortical areas of depressed patients. The BT prevented NOS expression during inflammation and ethanol-induced toxicity and oxidation (26). Moreover, the BT (30 and 100 mg/kg, IP) increased serotonin in the hypothalamus and hippocampus (1). It is clear that the central nitrergic and serotonergic systems are sensitive to BT. It can cross the blood-brain barrier by BT/GABA transporter-1 (BGT-1), accumulate in nervous tissues, and act as a

neurocognitive and neuroprotective agent (27).

It has been suggested that the antidepressant-like effects of E_2 are mediated through the inhibition of NOS, especially nNOS and NO/cGMP signaling pathways (5). The nitrergic system interacts with the serotoninergic system, and sex hormone depletion (OVX) led to an increase in NO, followed by a decrease in serotonin levels and finally depression. The present results suggest the possibility of involvement of the nitrergic and serotoninergic systems in the antidepressant effects of BT. However, there is scarce information about how BT interacts with NO and serotonin for its antidepressant activity.

It is worth mentioning that based on the limitations of the study, hippocampus NO, serotonin, as well as homocysteine levels could not be determined following the injection of the BT.

Based on the obtained results, OVX increased MDA but decreased SOD and GPx levels. The BT (25 and 50 mg/kg) decreased the MDA while increasing SOD and GPx. It has been reported that BT (1.5% of the total diet, orally) significantly increased catalase and GPx activity in oxidative stress induced by ethanol in the rat testes which can improve the antioxidant system against reactive oxygen species (ROS) (28). The BT has the ability to suppress ethanol consumption-induced oxidative stress in the brain, and it is useful against inflammation, oxidative stress for neurodegenerative disorders, and memory impairment (13). However, the direct mechanism for the antioxidant activity of BT is unclear. Recently, Hassanpour, Rezaei (29) has reported (10, 20, and 30 mg/kg) the anti-nociceptive and antioxidant activity of the BT in mice.The BT (0.163 mmol/kg) blocked a lipopolysaccharide-induced increase in mRNA expression of GAT2/BGT-1 which decreases the neuronal injury memory dysfunction in mice (13). Betaine-homocysteine methyltransferase is the enzyme that uses BT as a substrate, which mediates the transfer of a methyl group from BT to homocysteine and plays a key role in N-methyl-D-aspartate receptors, oxidative stress, and mitochondrial dysfunction (28).

The oxidation of homocysteine generates ROS via the prevention of homocysteine-induced toxicity by catalase leads to oxidative stress. Probably, the BT acts via the restoration of S-adenosyl methionine for the synthesis of glutathione and protects the cell against ROS (28). Di Pierro, Orsi (30) revealed that the antidepressant effects of the BT were partially mediated via the above-mentioned mechanisms in patients with mild-to-moderate depression.

In conclusion, these results suggest that the antidepressant activity of the BT is mediated via nitrergic and serotoninergic systems, as well as antioxidant activity in OVX mice. The potential significance of the BT suggests that it may have a substantial impact on neurophysiology and neuroprotection which merit that more studies are required to clarify the precise molecular mechanisms involved in its antidepressant effects.

Authors' Contribution

Study concept and design: Sh. H. and A. A
Acquisition of data: P. H. and Sh. H.
Analysis and interpretation of data: Sh. H.
Drafting of the manuscript: P. H.
Critical revision of the manuscript for important intellectual content: Sh. H.
Statistical analysis: P. H.
Administrative, technical, and material support: Sh. H. and A. A

Ethics

The present study was approved by the Ethics Committee of the Islamic Azad University, Tehran, Iran under the project number 45-5233-7841.

Conflict of Interest

The authors declare that they have no conflict of interest.

Acknowledgment

The authors thank the central laboratory of the Science and Research Branch, Islamic Azad University, Tehran, Iran, for their cooperation. This study was conducted as a part of the Doctor of Veterinary Medicine thesis of the first author.

References

1. Kim SJ, Lee L, Kim JH, Lee TH, Shim I. Antidepressant-like effects of lycii radicis cortex and betaine in the forced swimming test in rats. Biomol Ther (Seoul). 2013;21(1):79-83.
2. Albert KM, Newhouse PA. Estrogen, Stress, and Depression: Cognitive and Biological Interactions. Annu Rev Clin Psychol. 2019;15:399-423.
3. Newhouse P, Albert K. Estrogen, Stress, and Depression: A Neurocognitive Model. JAMA Psychiatry. 2015;72(7):727-9.
4. Albert K, Gau V, Taylor WD, Newhouse PA. Attention bias in older women with remitted depression is associated with enhanced amygdala activity and functional connectivity. J Affect Disord. 2017;210:49-56.
5. Heydarpour P, Salehi-Sadaghiani M, Javadi-Paydar M, Rahimian R, Fakhfouri G, Khosravi M, et al. Estradiol reduces depressive-like behavior through inhibiting nitric oxide/cyclic GMP pathway in ovariectomized mice. Horm Behav. 2013;63(2):361-9.
6. Amin B, Nakhsaz A, Hosseinzadeh H. Evaluation of the antidepressant-like effects of acute and sub-acute administration of crocin and crocetin in mice. Avicenna J Phytomed. 2015;5(5):458-68.
7. Hassanpour S, Zendehdel M, Babapour V, Charkhkar S. Endocannabinoid and nitric oxide interaction mediates food intake in neonatal chicken. Br Poult Sci. 2015;56(4):443-51.
8. Dhir A, Kulkarni SK. Nitric oxide and major depression. Nitric Oxide. 2011;24(3):125-31.
9. Gotti S, Martini M, Pradotto M, Viglietti-Panzica C, Panzica G. Rapid changes on nitrinergic system in female mouse hippocampus during the ovarian cycle. J Chem Neuroanat. 2009;38(2):117-23.
10. MacQueen G, Frodl T. The hippocampus in major depression: evidence for the convergence of the bench and bedside in psychiatric research? Mol Psychiatry. 2011;16(3):252-64.
11. Wegener G, Volke V. Nitric Oxide Synthase Inhibitors as Antidepressants. Pharmaceuticals (Basel). 2010;3(1):273-99.
12. Rajkumar R, Mahesh R. The auspicious role of the 5-HT3 receptor in depression: a probable neuronal target? J Psychopharmacol. 2010;24(4):455-69.
13. Miwa M, Tsuboi M, Noguchi Y, Enokishima A, Nabeshima T, Hiramatsu M. Effects of betaine on lipopolysaccharide-induced memory impairment in mice and the involvement of GABA transporter 2. J Neuroinflammation. 2011;8:153.
14. Ohnishi T, Balan S, Toyoshima M, Maekawa M, Ohba H, Watanabe A, et al. Investigation of betaine as a novel psychotherapeutic for schizophrenia. EBioMedicine. 2019;45:432-46.
15. Lin JC, Lee MY, Chan MH, Chen YC, Chen HH. Betaine enhances antidepressant-like, but blocks psychotomimetic effects of ketamine in mice. Psychopharmacology (Berl). 2016;233(17):3223-35.
16. Petronini PG, Alfieri RR, Losio MN, Caccamo AE, Cavazzoni A, Bonelli MA, et al. Induction of BGT-1 and amino acid system A transport activities in endothelial cells exposed to hyperosmolarity. Am J Physiol Regul Integr Comp Physiol. 2000;279(5):R1580-9.
17. Dadashpour Davachi N. Vasectomy in Mouse Model Using Electrosurgery Machine. Arch Razi Inst. 2019;74(2):191-5.
18. Dadashpour Davachi N. Effect of different surgical methods in the mouse embryo transfer: electrosurgery vs. cold surgical technique effects on repeated use of surrogate mothers, pregnancy rate, and post-surgical behavior. Vet Res Forum. 2021.
19. Kalbasi Anaraki D, Sianati S, Sadeghi M, Ghasemi M, Paydar MJ, Ejtemaei Mehr S, et al. Modulation by female sex hormones of the cannabinoid-induced catalepsy and analgesia in ovariectomized mice. Eur J Pharmacol. 2008;586(1-3):189-96.
20. Sadeghi M, Sianati S, Anaraki DK, Ghasemi M, Paydar MJ, Sharif B, et al. Study of morphine-induced dependence in gonadectomized male and female mice. Pharmacol Biochem Behav. 2009;91(4):604-9.
21. Castagne V, Moser P, Roux S, Porsolt RD. Rodent models of depression: forced swim and tail suspension behavioral despair tests in rats and mice. Curr Protoc Neurosci. 2011;Chapter 8:Unit 8 10A.
22. Cryan JF, Mombereau C, Vassout A. The tail suspension test as a model for assessing antidepressant

activity: review of pharmacological and genetic studies in mice. Neurosci Biobehav Rev. 2005;29(4-5):571-625.

23. Steru L, Chermat R, Thierry B, Simon P. The tail suspension test: a new method for screening antidepressants in mice. Psychopharmacology (Berl). 1985;85(3):367-70.
24. Donato F, de Gomes MG, Goes AT, Filho CB, Del Fabbro L, Antunes MS, et al. Hesperidin exerts antidepressant-like effects in acute and chronic treatments in mice: possible role of l-arginine-NO-cGMP pathway and BDNF levels. Brain Res Bull. 2014;104:19-26.
25. Zhang J, Huang XY, Ye ML, Luo CX, Wu HY, Hu Y, et al. Neuronal nitric oxide synthase alteration accounts for the role of 5-HT1A receptor in modulating anxiety-related behaviors. J Neurosci. 2010;30(7):2433-41.
26. Zhao G, He F, Wu C, Li P, Li N, Deng J, et al. Betaine in Inflammation: Mechanistic Aspects and Applications. Front Immunol. 2018;9:1070.
27. Knight LS, Piibe Q, Lambie I, Perkins C, Yancey PH. Betaine in the Brain: Characterization of Betaine Uptake, its Influence on Other Osmolytes and its Potential Role in Neuroprotection from Osmotic Stress. Neurochem Res. 2017;42(12):3490-503.
28. Alirezaei M, Jelodar G, Ghayemi Z. Antioxidant Defense of Betaine Against Oxidative Stress Induced by Ethanol in the Rat Testes. Int J Pept Res Ther. 2012;18(3):239-47.
29. Hassanpour S, Rezaei H, Razavi SM. Anti-nociceptive and antioxidant activity of betaine on formalin- and writhing tests induced pain in mice. Behav Brain Res. 2020;390:112699.
30. Di Pierro F, Orsi R, Settembre R. Role of betaine in improving the antidepressant effect of S-adenosyl-methionine in patients with mild-to-moderate depression. J Multidiscip Healthc. 2015;8:39-45.