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

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Abstract

The aim of current study was to determine anti-depressant effect of betaine (BT) in ovariectomized mice and its possible interaction with nitrergic and serotonergic systems. In experiment 1, mice kept as control and sham groups, ovariectomized (OVX), OVX+BT (12.5mg/kg), OVX+BT (25mg/kg) and OVX+BT (50mg/kg). In experiment 2, mice kept as control and sham, OVX, OVX+BT (50mg/kg), OVX+L-NAME (10mg/kg) and OVX+BT and L-NAME. Experiments 3-5 were similar to experiment 2, except L-Arginine (50mg/kg), Fluoxetine (5mg/kg) and Cyproheptadine (4mg/kg) were injected instead of the L-NAME. Then forced swimming test (FST), tail suspension test (TST) and open field test (OFT) tests were done. Also, serum Malondialdehyde (MDA), Superoxide dismutase (SOD), Glutathione peroxidase (GPx) and total antioxidant status (TAS) levels were determined. According to the findings, OVX increased immobility time compared to control group (P<0.05). BT (50mg/kg) decreased depression induced immobility time compared to OVX group (P<0.05). Co-injection of the BT+L-NAME decreased depression induced immobility time in TST and FST and increased number of crossing in OFT (P<0.05). Co-injection of the BT+L-Arginine significantly diminished antidepressant activity of the BT on immobility time and decreased positive effect of the BT on number of crossing (P<0.05). Co-injection of the BT+fluoxetine significantly amplified antidepressant activity of the BT on immobility time and number of crossing (P<0.05). Co-injection of the BT+cyproheptadine decreased antidepressant activity of the BT on immobility time and number of crossing (P<0.05). BT (25 and 50mg/kg) reduced the MDA while elevated SOD and GPx levels in OVX
mice (P<0.05). It seems, antidepressant activity of the BT mediates via nitrergic and serotoninergic systems in OVX mice.

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

**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 (Kim et al. 2013). Major depressive disorder (MDD) is complex which impresses brain physiological function and alters emotional and cognitive processes. The incidence and prevalence of the MDD is higher in women than men because of hormonal fluctuations and increases in the menopausal stage (Albert and Newhouse, 2019). Ovarian hormones have a prominent role in emotional perception, mood regulation, response to stress and cognition. Alterations in estradiol milieu during the menstrual cycle, parturition and menopause, increase depression risk and mood disruption in women (Newhouse and Albert 2015). Bilaterally OVX in mice leads to depressive-like behavior (Albert et al. 2017) and estradiol therapy showed antidepressant-like effects in OVX mice (Heydarpour et al. 2013). Although the direct mechanism for effects of estradiol in mood and depression is not fully elicited, it acts by modulation of the dopaminergic, serotonergic and nitric oxide systems (Heydarpour et al. 2013; Amin et al. 2015).

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 (Hassanpour et al. 2015). Nitric oxide has crucial role in pathogenesis of depression by its modulatory effects on serotonin, dopamine and norepinephrine (Dhir and Kulkarni, 2011). The fluctuations of the NO concentrations in the hippocampus during the ovarian cycle may suggest that the nitrergic system has a mediatory role on effects of estrogen (Gotti et al. 2009). The hippocampus plays a key role in MDD (Macqueen and Frodl, 2011). Estrogen deficiency leads to increased hippocampus NO levels and 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 (Wegener and Volke, 2010). Based on evidence, nitrergic neurons have synapses with serotoninergic neurons through which NO modulates serotonin release, reuptake and function as well as its extracellular levels (Walia and Gilhotra, 2016). Serotonin is the main neurotransmitter for the regulation of mood and its deficiency leads to depression (Rajkumar and Mahesh, 2010).
There are several antidepressant drugs for pathogenesis of the MDD for example selective serotonin reuptake inhibitors and tricyclic anti-depressants which affect the monoaminergic system (Adongo et al. 2015). However, sedation, seizures and sexual dysfunction are major side effects these antidepressants (Adongo et al. 2015). Therefore, there is growing interest in new antidepressant drugs from natural and bioactive products. Betaine (glycine betaine or trimethylglycine) is a natural product, primary isolated from the Beta vulgaris plant and other plants and microorganisms. Betaine can inhibit nuclear factor-κB (NF-κB) and cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS) and tumor necrosis factor (TNF-α) (Miwa et al. 2011). Betaine has positive effect against biological stresses and its levels decreased in schizophrenia patients (Ohnishi et al. 2019). Also, has positive effect against memory impairment in mice (Miwa et al. 2011). Immobility time in FST resembles a state of despair. TST and FST are useful methods for depression-like behavioral in rodents. This tests reflects depressive behavior in humans (Walia and Gilhotra, 2016). Betaine played a major role in improving individuals with mild-to-moderate depression (Lin et al. 2016). In the hippocampus and hypothalamus, BT alters serotonin levels in FST (Kim et al. 2013). and its injection reduced the immobility time in rats (Petronini et al. 2000). Despite it has antidepressant effects, but there is no information about its antidepressant activity with sex hormone deficiency-related depression. Thus, the aim of this paper was to determine antidepressant effects of the BT in OVX mice and its possible interaction with the nitrergic and serotoninergic systems.

**Materials and Methods**

**Animals**

A total of 240 adult female NMRI mice (weigh 28–30 g and age 8–10 weeks old) were supplied from the Pasteur Institute (Tehran, Iran) and kept at physiology laboratory of Science and Research Branch, Islamic Azad University (Tehran, Iran) according to Guide for the Care and Use of Laboratory Animals by the US National Institutes of Health (NIH Publication No. 85–23, revised 1996) (Zimmermann, 1983).

**Drugs**

Betaine, 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).
**Experimental OVX**

Following anesthesia by intraperitoneal (i.p.) injection of ketamine hydrochloride (50 mg/kg) and xylazine hydrochloride (5 mg/kg) (Alfasan, Woerden, Holland), the lumbar dorsum was shaved and exposed skin was scrubbed by a sterile saline wipe and by 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 nonabsorbable). In the sham group, the parovarian fatty tissues and ovaries were just retracted and replaced (Kalbasi Anaraki et al. 2008; Sadeghi et al. 2009). All behavioral tests were done 10 days after recovery (Heydarpour et al. 2013).

**17β-estradiol assay**

Blood samples were taken from each mouse via cardiac puncture for 17β-estradiol (E2) by direct and competitive chemiluminescence immunoassay detection by the LIASION Estradiol (310400) kit.

**Experimental procedure**

Then OVX mice were randomly allocated into 5 experimental groups (n=48 in each experiment and 8 mice in each group). Experiment 1 included (A) control group without surgery and injected with saline (10 ml/kg) 1 hour before the test, (B) sham group which had no OVX and was injected with saline (10 ml/kg) 1 hour before the test, (C) OVX then i.p injected with saline (10 ml/kg) 1 hour before the test, (D) OVX mice injected with BT (12.5 mg/kg) 1 hour before the test, (E) OVX mice injected with BT (25 mg/kg) 1 hour before the test and (F) mice which were OVX then i.p injected with BT (12.5 mg/kg) 1 hour before to the test. In experiment 2, group (A) control without surgery and was injected with saline (10 ml/kg) 1 hour to prior the test, (B) sham group with no ovariectomy (OVX) and injected with saline (10 ml/kg) 1 hour before the test, group (C) was OVX then i.p injected with saline (10 ml/kg) 1 hour before the test, group (D) OVX mice injected with BT (50 mg/kg) 1 hour before the test, (E) OVX mice injected with L-NAME (10mg/kg) 1 hour before the test and group (F) OVX mice injected with BT (50 mg/kg) 1 hour prior to the test; 15 minutes after the final injection animals were i.p injected with L-NAME (10mg/kg) and 45 minutes later tests were done. Experiment 3 consisted of the following groups: (A) control without surgery and injected with saline (10 ml/kg) 1 hour before the test, (B) sham with no OVX and injected with saline (10 ml/kg) 1 hour before the test, (C) OVX mice injected with saline (10 ml/kg)1 h prior to the test, (D) OVX animal injected with BT (50 mg/kg) 1 hour before the test, (E) OVX mice injected with L-Arginine (50mg/kg) 1 hour before the test and (F) OVX mice injected with BT (50 mg/kg).
1 hour before the test and 15 minutes later i.p injected with L-arginine (50mg/kg) and 45 minutes later tests were done. In experiment 4, group (A) control without surgery and injected with saline (10 ml/kg) 1 hour before the test, group (B) sham treatment with no OVX and injected with saline (10 ml/kg)1 hour prior to the test, group (C) was OVX then i.p injected with saline (10 ml/kg) 1 hour before the test, group (D) OVX mice injected with BT (50 mg/kg) 1 hour before the test and group (E) were OVX mice injected with fluoxetine (5mg/kg) 1 hour before the test and group (F) OVX mice injected with BT (50 mg/kg) 1 hour before the test, and 15 minutes after the final injection the animals were i.p injected with fluoxetine (5mg/kg) and 45 minutes later tests were done. In experiment 5, group (A) control without surgery and injected with saline (10 ml/kg) 1 hour before the test, (B) sham with no OVX and injected with saline (10 ml/kg) 1 hour before the test, (C) OVX mice injected with saline (10 ml/kg) 1 hour before the test, (D) was OVX animal injected with BT (50 mg/kg) 1 hour prior to the test, group (E) OVX mice injected with cyproheptadine (4mg/kg) 1 hour before the test and (F) included OVX mice injected with BT (50 mg/kg) 1 hour before the test and 15 minutes after the final injection the animals were i.p injected with cyproheptadine (4mg/kg) and 45 minutes later the FST, TST and OFT tests were done. At the end of the study, serum MDA, SOD, GPx and TAS levels were determined.

**Forced Swimming Test**

The FST was done using the described protocol in mice (Castagné et al. 2011). Animal was plunged into a glass cylinder containing 25 ± 1 °C water for 15 minutes (pre-test session). 24 hours later, the test was repeated for a 6 minutes’ period (test session). When mouse ceased struggling and remained floating motionless in the water, the immobility time recorded as total period of immobility during the last 4 minutes’of the 6 minutes’.

**Tail Suspension Test**

It is knowns as common antidepressant-like activity in mice (Cryan et al. 2005). According to the Steru et al. (1985). 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 6 minutes.

**Open field test**

The OFT was used to determine the possible effects of the BT on the locomotor and exploratory activities. The open-field was done using a 45x45x30 cm³ poly wood cage. The floor of the open field cage was divided by masking tape markers into 3x3 cm² squares. Mice was placed
individually observed for 6 min for number of segments crossed with the four paws (Donato et al. 2014).

**Antioxidant activity**

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

**Statistical analysis**

Data was 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. Data is presented as the mean ± SEM and $p < 0.05$ were considered to indicate significant difference.

**Results**

The results of the anti-depressant and antioxidant effects of the BT in ovariectomized 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 (Heydarpour et al. 2013). As seen in figure 1, no significant differences were observed on immobility time in the FST and TST tests in the sham versus control groups ($P > 0.05$). The OVX significantly increased immobility time in FST and TST tests compared to the control group ($P < 0.05$). Betaine (12.5 mg/kg) had no effect on immobility time ($P > 0.05$) while dosage of 25 and 50 mg/kg decreased depression induced immobility time in comparison to the OVX group ($P < 0.05$) in a dose dependent manner. There was no significant difference in the number of crossings in the OFT between sham and control mice ($P > 0.05$). The OVX significantly decreased the number of crossing in the OFT compared to the control group ($P < 0.05$). Betaine 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 in comparison to OVX mice ($P < 0.05$).

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

According to the results, immobility time significantly increased in the OVX group compared to the control group (P<0.05). Betaine (50 mg/kg) significantly lessened immobility time in comparison to OVX mice (P<0.05). The L-arginine (50 mg/kg) had no effect on immobility time compared to OVX mice (P>0.05). 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 OVX mice (P<0.05). 50 mg/kg of the BT significantly increased the number of crossings in comparison to OVX mice (P<0.05). 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 in comparison to the OVX group (P<0.05). It seems that the antidepressant activity of the BT is mediated via the nitrergic system in ovariectomized mice (figure 3).

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

As shown in figure 5, OVX significantly increased immobility time in the FST and TST tests compared to the control group (P<0.05). Betaine (50 mg/kg) significantly reduced depression induced immobility time in comparison to OVX mice (P<0.05). The cyproheptadine (4 mg/kg) had no effect on immobility time compared to the OVX group (P>0.05). 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 OVX mice (P<0.05). BT (50 mg/kg) significantly increased the number of crossings compared to the OVX group (P<0.05). Co-injection of the
BT (50 mg/kg) + cyproheptadine (4 mg/kg) decreased the positive effect of the BT on the number of crossings in comparison to the OVX mice ($P<0.05$). This may suggest that the antidepressant activity of the BT is mediated via the serotonergic system in ovariectomized mice.

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

**Discussion**

The effects of the gonadal E\textsubscript{2} on emotions and mood, depression and cognitive behavior are complex and differ during the reproductive life stage. In women with MDD vulnerability, E\textsubscript{2} may support the healthy functioning of these areas in the brain whereas low E\textsubscript{2} levels increase the risk for depressive episodes (Newhouse and Albert, 2015). Rodents are ideal models for depressive-like states and the strain, age at OVX and time of behavioral test following OVX influence the results (Heydarpour et al. 2013). In our study, the serum E\textsubscript{2} levels on adult female NMRI mice below 20±2 pg/ml were taken as OVX which was similar to previous reports (Heydarpour et al. 2013; Kim et al. 2013). It has been reported that E\textsubscript{2} decreases the latency to beginning of antidepressants in TST and FST. Even though the direct mechanism for antidepressant-like actions of E\textsubscript{2} is not well-understood, its effects are mediated via involving E\textsubscript{2} receptors (ER\textalpha{} and ER\textbeta{}), serotonergic, dopaminergic and noradrenergic receptors in the amygdala and hippocampus (Dhir and Kulkarni, 2008). Also, E\textsubscript{2} modulates neural NOS (nNOS) expression by activating ER\textbeta{} in hippocampal neurons. Additionally, E\textsubscript{2} modulates serotonin receptor mRNA levels in a genetic model of depression in mice (Heydarpour et al. 2013). Finally, serotonin suppresses nNOS expression in the hippocampus in anxiety-related behavior (Zhang et al. 2010). Nitric oxide has a key role in the pathogenesis of the MDD and its levels increase in the hippocampus of these patients (Dhir and Kulkarni, 2011). In this regard, Heydarpour et al. (2013) reported that the non-selective NOS inhibitor (L-NAME, 30 mg/kg, i.p.) decreased immobility time in OVX mice. Also, L-NAME (10 mg/kg), 15 minutes after a sub-effective dose of the E\textsubscript{2} (1 μg/kg) had positive antidepressant-like effect in OVX mice which indicating for involvement of hippocampal NO signaling in the antidepressant
Our present findings suggest that OVX increased immobility time and BT (25 and 50mg/kg) decreased depression-induced immobility time in FST and TST tests. A sub-effective dose of L-NAME (10 mg/kg) had no effect on immobility time but co-injection of the BT + L-NAME decreased depression-induced immobility time in OVX mice. Also, a sub-effective dose of the NO processor (L-Arginine, 50 mg/kg) had no effect on immobility time but co-injection of the BT and L-arginine significantly decreased the antidepressant activity of the BT on immobility time in OVX mice. On the other hand, 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 tests while 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 but co-injection of the BT and cyproheptadine decreased the antidepressant activity of the BT in OVX mice. In a previous report, Kim et al. (2013) reported that BT (30 and 100 mg/kg, i.p.) significantly decreased immobility time in FST and our results were similar to this report. Also, they reported that the effect of the BT (30 mg/kg) was quite close to fluoxetine, 10 mg/kg (as control) (Kim et al. 2013). Betaine played a major role in improving individuals suffering from mild-to-moderate depression (Lin et al. 2016) injection of 30 and 100 mg/kg of which decreased immobility time on FST in rats (Petronini et al. 2000). Most antidepressants act by increasing serotonin and norepinephrine levels in the hippocampus, limbic, thalamic and prefrontal cortical area of depressed patients. Betaine prevented NOS expression during inflammation and ethanol-induced toxicity and oxidation (Zhao et al. 2018). Also, BT (30 and 100 mg/kg, i.p.) increased serotonin in the hypothalamus and hippocampus (Kim et al. 2013). 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) and accumulate in nervous tissues and act as a neurocognitive and neuroprotective agent (Knight et al. 2017). It has been suggested antidepressant-like effects of E2 are mediated through inhibition of NOS, especially nNOS, and NO/cGMP signaling pathways (Heydarpour et al. 2013). The nitrergic system interacts with the serotonergic system and sex hormone depletion (OVX) led to increase NO and decreased serotonin levels and finally depression. The present results suggest the possibility of involvement of the nitrergic and serotonergic systems in the antidepressant effects of the BT. However, there is scarce information about how BT interacts with NO and serotonin for its antidepressant activity. Also, based on the limitations of the study, we were not able to determine
hippocampus NO and serotonin as well as homocysteine levels following the injection of the BT.

Based on the obtained results, OVX increased MDA and decreased SOD and GPx levels. Betaine (25 and 50mg/kg) decreased the MDA while increasing SOD and GPx. It has been reported that BT (1.5 % of 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) (Alirezaei et al. 2012). Betaine has the ability to suppress ethanol consumption-induced oxidative stress in the brain. Betaine is useful against inflammation, oxidative stress for neurodegenerative disorders and memory impairment (Miwa et al. 2011). However, the direct mechanism for the antioxidant activity of BT is unclear. Recently, Hassaanpour et al. (2020) reported BT (10, 20 and 30 mg/kg) has anti-nociceptive and antioxidant activity in mice. Betaine (0.163 mmol/kg) blocked a lipopolysaccharide-induced increase in mRNA expression of GAT2/BGT-1 which decreases neuronal injury memory dysfunction in mice (Miwa et al. 2011). Betaine-homocysteine methyltransferase (BHMT) 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 for N-methyl-D-aspartate receptors, oxidative stress and mitochondrial dysfunction (Alirezaei et al. 2012). Oxidation of homocysteine generates ROS via the prevention of homocysteine-induced toxicity by catalase, leading to oxidative stress. Perhaps, BT acts via restoration of S-adenosyl methionine for the synthesis of glutathione and protects the cell against ROS (Alirezaei et al. 2012). For instance, Di Pierro et al. (2015) revealed that the antidepressant effects of the BT are 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 serotonergic 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 research is needed to clarify the precise molecular mechanisms involved in its antidepressant effects.

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References


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

<table>
<thead>
<tr>
<th>Group</th>
<th>MDA (nmol/ml)</th>
<th>SOD (IU/ml)</th>
<th>GPx (IU/ml)</th>
<th>TAS (nmol/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.86 ± 0.24⁴</td>
<td>65.84 ± 2.13³</td>
<td>6.24 ± 0.26⁴</td>
<td>1.85 ± 0.02⁴</td>
</tr>
<tr>
<td>Sham</td>
<td>6.01 ± 0.32³</td>
<td>53.50 ± 2.11³</td>
<td>6.35 ± 0.16⁴</td>
<td>1.84 ± 0.01⁴</td>
</tr>
<tr>
<td>OVX</td>
<td>12.31 ± 0.11⁴</td>
<td>17.66 ± 1.11³</td>
<td>2.23 ± 0.11⁴</td>
<td>1.80 ± 0.04⁴</td>
</tr>
<tr>
<td>Betaine (12.5 mg/kg)</td>
<td>11.58 ± 0.25⁴</td>
<td>17.65 ± 1.16³</td>
<td>2.17 ± 0.23³</td>
<td>1.82 ± 0.02³</td>
</tr>
<tr>
<td>Betaine (25 mg/kg)</td>
<td>8.46 ± 0.13⁵</td>
<td>31.66 ± 1.21³</td>
<td>3.42 ± 0.16³</td>
<td>1.83 ± 0.03³</td>
</tr>
<tr>
<td>Betaine (50 mg/kg)</td>
<td>6.56 ± 0.24⁴</td>
<td>51.53 ± 1.18³</td>
<td>4.54 ± 0.11³</td>
<td>1.81 ± 0.01³</td>
</tr>
</tbody>
</table>

OVX: ovariectomy, MDA: malondialdehyde, SOD: superoxide dismutase, GPx: glutathione peroxidase, TAS: total antioxidant status. Different letters (a-d) indicate significant differences between treatments (P<0.05), (n=10 per group).
Figure 1. Effects of Betaine (12.5, 25 and 50 mg/kg) on FST (A), TST (B) and OFT (C) tests in ovariectomized mice. Different letters (a-c) indicate significant differences between treatments (P<0.05).

TST: tail suspension test, FST: forced swimming test, OFT: open field test.
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) tests in ovariectomized mice. Different letters (a-c) indicate significant differences between treatments (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.
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) tests in ovariectomized mice. Different letters (a-d) indicate significant differences between treatments (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) tests in ovariectomized mice. Different letters (a-d) indicate significant differences between treatments ($P<0.05$). 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) tests in ovariectomized mice. Different letters (a-d) indicate significant differences between treatments ($P<0.05$). TST: tail suspension test, FST: forced swimming test, OFT: open field test.