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Evaluation the anti-depressant effect of metformin in ovariectomized mice: possible involvement of nitrergic and serotoninergic systems

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Abstract

Background and Objective: Depressive disorders are the most prevalent form of the mental disorders in postmenopausal and there is information for beneficial effects of the metformin (MET) in depression. However, there is no report on anti-depressant effect of the MET during postmenopausal. Thus, the aim of study was to determine anti-depressant effect of the MET in ovariectomized (OVX) mice and possible involvement with nitrergic and serotoninergic systems.

Materials and Methods: Two hundred and fifty female NMRI mice randomly allocated into 5 experimental groups (each with 5 groups [A-E], n=50). In experiment 1, mice allocated as (A) control, (B) OVX injected with saline, (C) OVX injected with MET (100 mg/kg), (D) OVX mice injected with MET (200 mg/kg), (E) OVX injected with MET (400 mg/kg) at 24, 6 and 1 h prior the test. In experiment 2, experimental grouping was (A) control, (B) OVX injected with saline, (C) OVX mice injected with MET (400mg/kg), (D) OVX mice injected with L-NAME (10mg/kg), (E) OVX mice injected with MET (400mg/kg) + L-NAME (10mg/kg). Experiments 3-5 were similar to experiment 2, except OVX mice injected with L-arginine (50mg/kg), cyproheptadine (4mg/kg) and fluoxetine (5mg/kg) instead of L-arginine. Following injections forced swimming test (FST), tail suspension test (TST) and open field test (OFT) were done. At the end of the study, serum malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (GPx) and total antioxidant status (TAS) were determined.

Results: According to the results, MET (200 and 400mg/kg) decreased immobility time in TST and FST ($P<0.05$). Co-injection of the MET+L-NAME decreased immobility time in TST and FST ($P<0.05$). Co-injection of MET+L-Arginine significantly diminished antidepressant activity of the MET ($P<0.05$). MET + cyproheptadine decreased antidepressant activity of the MET ($P<0.05$). MET + fluoxetine significantly amplified antidepressant activity of the MET ($P<0.05$). MET (200 and 400mg/kg) improved serum MDA, SOD and GPx levels ($P<0.05$).

Conclusion: It seems antidepressant activity of the MET mediates by nitrergic and serotoninergic system in OVX mice.

Keywords: Depression, Ovariectomy, Metformin, Nitric oxide, Serotonin, Antioxidant, Estradiol deficiency, Mice

1. Introduction

Depressive disorders are the most prevalent form of mental illness (1). Major depression disorder characterized by change in psychosocial and physical impairment mood as well as lack of interest in the surroundings (2). The role of ovarian hormones on regulation of affective disorders

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has been established and it is known that abrupt oscillations of the gonadal hormones throughout their reproductive life precipitate or exacerbate episodes of depression or anxiety (3). Sex hormones play key role in depression among menopausal women (4). Lower estrogen levels are responsible for incidence of depression risk in post-menopausal women (5). Animal studies were also supported for depression in the OVX rodents (6). Fluctuations in estradiol, follicle-stimulating hormone (FSH) and luteinizing hormone (LH) levels is correlate with the worsening of depression during the premenopausal period (7). Estrogen therapy can prolong the immobility time in FST as a support for anti-depressive properties of the estrogen (3, 8). Metformin is a classic medication for the treatment of Type 2 diabetes. It has beneficial for insulin resistance and regulating glucose metabolism, decrease reactive protein, promoting endothelial nitric oxidase synthesis (eNOS) expression and nitric oxide (NO) production (9). Depressive symptoms are common in patients with diabetes and MET is beneficial to decrease depressive symptoms in these patients (10). It is revealed plasma NO level is higher in depressed patients (11). L-N-arginine methyl ester or NG-nitro-L-arginine methyl ester (L-NAME), a type of general NOS inhibitor which can decrease immobility time in FST and this effect reversed by pre-treatment with L-arginine (the NOS substrate) (12). Acute and chronic treatment with L-NAME has anti-depressive-like response in FST (13). Also, NOS inhibitor elicited anti-depressive-like effect in FST. NG-nitro-L-arginine as non-preferential NOS inhibitor has antidepressant like effects in FST and augmented the effect of antidepressants (14).

Based on reports, little information exists regarding the antidepressant properties of the MET but there is no report for antidepressant activity of the MET in OVX mice. Thus, the primary aim of the current study was to determine antidepressant effects of the MET and secondary its possible interaction with the nitrergic and serotoninergic systems in OVX mice.

2. Materials and Methods

2.1. Animals

A total of 250 adult female NMRI mice 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) (15) (IR.IAU.SRB.REC.1399.1125).

2.2. Drugs

Metformin, L-NAME (nitric oxide inhibitor, 10mg/kg), L-Arginine (nitric oxide processor, 50mg/kg), Cyproheptadine (serotonergic receptor antagonist, 4mg/kg) and Fluoxetine (selective serotonin reuptake inhibitor, 5mg/kg) were purchased from Sigma Aldrich, (St, USA).

2.3. Ovariectomy

Anesthesia was induced by intraperitoneal injection of Ketamine (50 mg/kg) and Xylazine (5 mg/kg) (Alfasan, Woerden, Holland). After the onset of anesthesia, the lumbar dorsum was shaved, and the exposed skin prepared for aseptic surgery (a 10% povidene-iodine scrub followed by a sterile saline wipe). Surgery was performed as previously described (16, 17). In brief, skin was opened with a 1-2 cm incision in the midline on the lumbar vertebral line. About 1 cm to each flank, parovarian fatty tissue was identified and pulled out through a small incision. The exposed ovary and associated oviduct were removed. Then the skin incision was sutured (4-0 nonabsorbable). In the sham-operated animals, the parovarian fatty tissues and ovaries were just retracted and replaced. All behavioral tests were initiated after a recovery period of 10 days (18).

2.4. Experimental procedure

Then OVX mice randomly allocated into 5 experiments (each with 5 groups [A-E], n=50). In experiment 1, mice received as (A) control group without surgery and injected with saline (10 ml/kg) at 24, 6 and 1 h prior the test, (B) mice first get OVX and then i.p injected with saline (10 ml/kg) at 24, 6 and 1 h prior the test, (C) OVX mice i.p injected with MET (100 mg/kg) at 24, 6 and 1 h prior the test, (D) OVX mice injected with MET (200 mg/kg) at 24, 6 and 1 h prior the test, (E) OVX mice i.p injected with MET (400 mg/kg) at 24, 6 and 1 h prior the test. In experiment 2, groups were: (A) control without surgery and injected saline (10 ml/kg) at 24, 6 and 1 h prior the test, (B) OVX mice i.p injected with saline (10 ml/kg) at 24, 6 and 1 h prior the test, (C) OVX mice injected with MET (400mg/kg), (D) OVX mice injected with L-NAME (10mg/kg), (E) OVX mice injected with MET (400mg/kg) at 24, 6 and 1 h prior the test, 15 minutes after final injection of the MET, i.p injected with L-NAME (10mg/kg). In experiments 3-5 were similar to experiment 2, except mice treated with L-arginine (50mg/kg), cyproheptadine (4mg/kg) and fluoxetine (5mg/kg) instead of the L-NAME (10mg/kg). Then FST, TST and OFT were done and finally serum MDA, SOD, GPx and TAS levels were determined.

2.5. Forced swimming test (FST)

One hour after last treatment of acute study or forty-eight hours after tail-suspension testing, mice were gently placed in a Plexiglas cylindrical tank (15 cm in diameter), filled with 35 cm of room-temperature water to ensure that animals could not touch the bottom of the container with their hind paws or their tails. The animals were removed, dried and returned to their home cages after 15 min in water (pre-test). They

were again placed in the cylinder 24 h later. The total duration of immobility was measured during a 6-minute period. Mice were considered immobile if they made no attempts to escape and remained floating with their heads above the water. A decrease in the duration of immobility is an indicator of antidepressant-like effect (19).

2.6. Tail suspension test (TST)

Animals were suspended on a horizontal beam (height 33 cm) using adhesive tape wrapped around the tip of the tail. The time spent immobile was recorded during 6- minute testing (20).

2.7. Open Field test (OFT)

Before performing the forced swimming test (FST), the locomotors behavior was accessed through an open field test (OFT) as described previously. In short, the apparatus consists of a wooden box (40×60×50 cm3), the floor area of which was divided into 12 equal rectangles. Each mouse after placing within the center of the arena, the number of rectangles was counted covered with all paws while moving within the box for at least 6 minutes (21).

2.8. Antioxidant activity

At the end of the tests, blood samples were collected via cardiac puncture and serum MDA, SOD, GPx and total antioxidant capacity (TAC) were determined by ELISA method using Zell Bio GmbH (Germany) commercial assay kits.

2.9. 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 ± SE and p <0.05 were considered to indicate significant difference.

3. Results

According to experiment 1, OVX increased immobility time in FST compared to the control group (P<0.05). MET in a dose dependent manner decreased immobility time compared to the control and OVX group (P<0.05) (figure 1A, B). Also, MET significantly increased the number of crossings in the OFT compared to OVX group (P<0.05) (figure 1C).

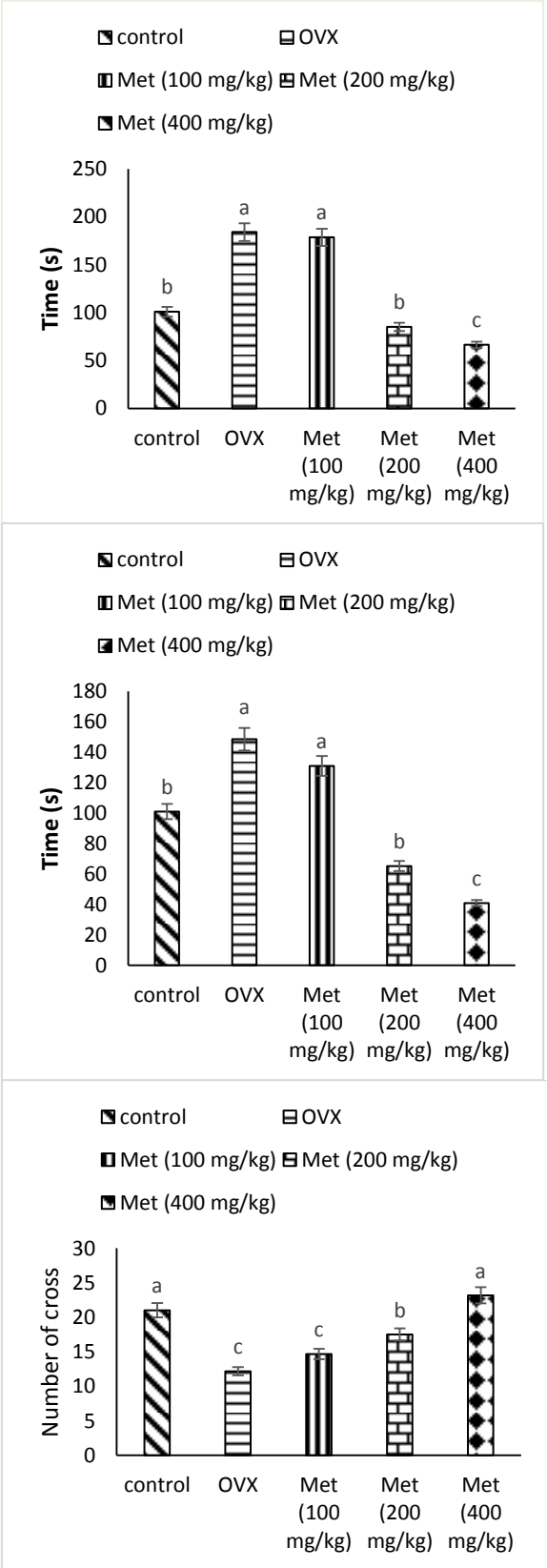
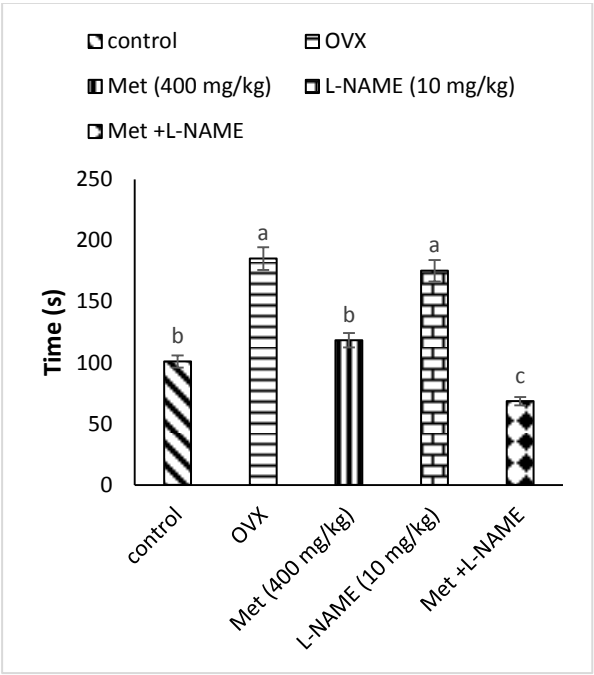
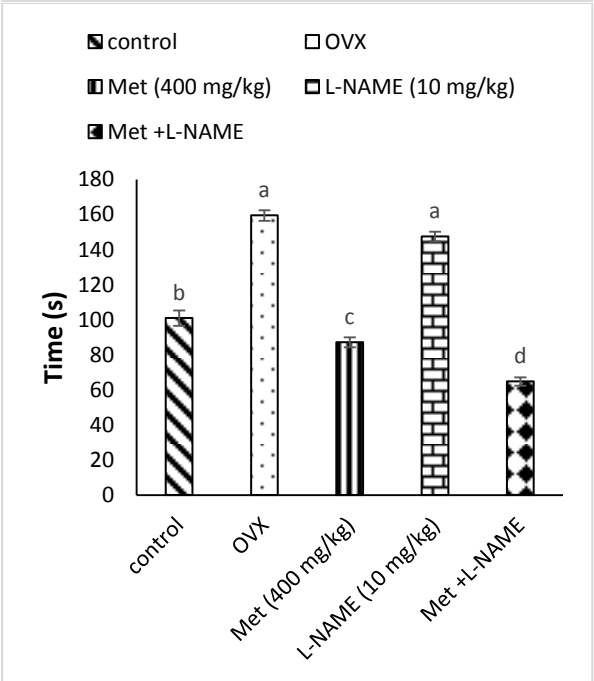


Figure 1. Effects of Metformin (100, 200 and 400 mg/kg) on FST (up), TST (middle) and OFT (down) in ovariectomized mice. Different letters (a-c) indicate significant differences between treatments (P<0.05). Met: Metformin, OVX: ovariectomy, TST: tail suspension test, FST: forced swimming test, OFT: open field (n=50).

Based on experiment 2, MET (400 mg/kg) decreased immobility time compared to the OVX-untreated rat (P<0.05). L-NAME (10 mg/kg) had no effect on immobility time (P>0.05). Co-injection of the L-NAME and MET significantly decreased immobility time using FST (P<0.05) (figure 2A). Co-injection of the L-NAME and MET significantly decreased immobility time using TST (P<0.05) (figure 2B). L-NAME + MET significantly increased the number of crossings in the OFT in comparison to OVX mice (P<0.05) (figure 2C).





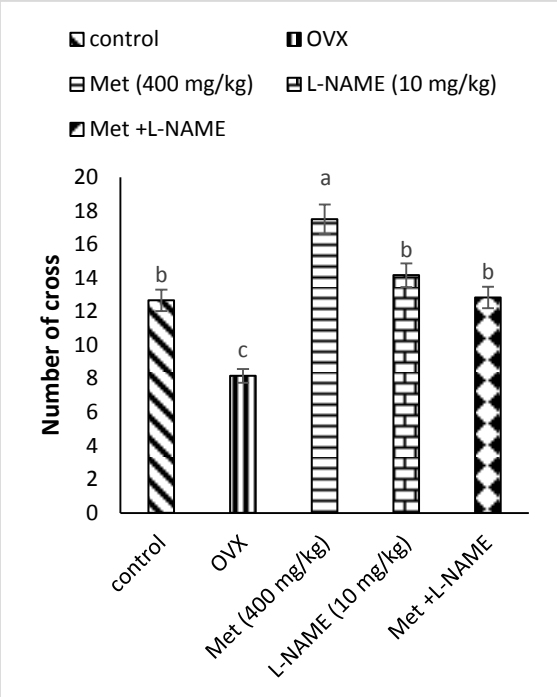


Figure 2. Effects of Metformin (400 mg/kg), L-NAME (10 mg/kg) and their co-injection on FST (up), TST (middle) and OFT (down) in ovariectomized mice. Different letters (a-c) indicate significant differences between treatments (P<0.05). Met: Metformin, OVX: ovariectomy, L-NAME: L-NG-Nitro arginine methyl ester (L-NAME), TST: tail suspension test, FST: forced swimming test, OFT: open field (n=50).

As seen in experiment 3, OVX increased immobility time using FST compared to the control group (P<0.05). L-arginine (50 mg/kg) had no effect on immobility time (P>0.05). Co-injection of the L-arginine and MET (400 mg/kg) increased immobility time using FST compared to MET (P<0.05) (figure 3A). L-arginine + MET (400 mg/kg) increased immobility time in TST (P<0.05) (figure 3B). Also, L-arginine + MET (400 mg/kg) increased the number of crossings in the OFT in comparison to OVX mice (P<0. 05) (figure 3C).

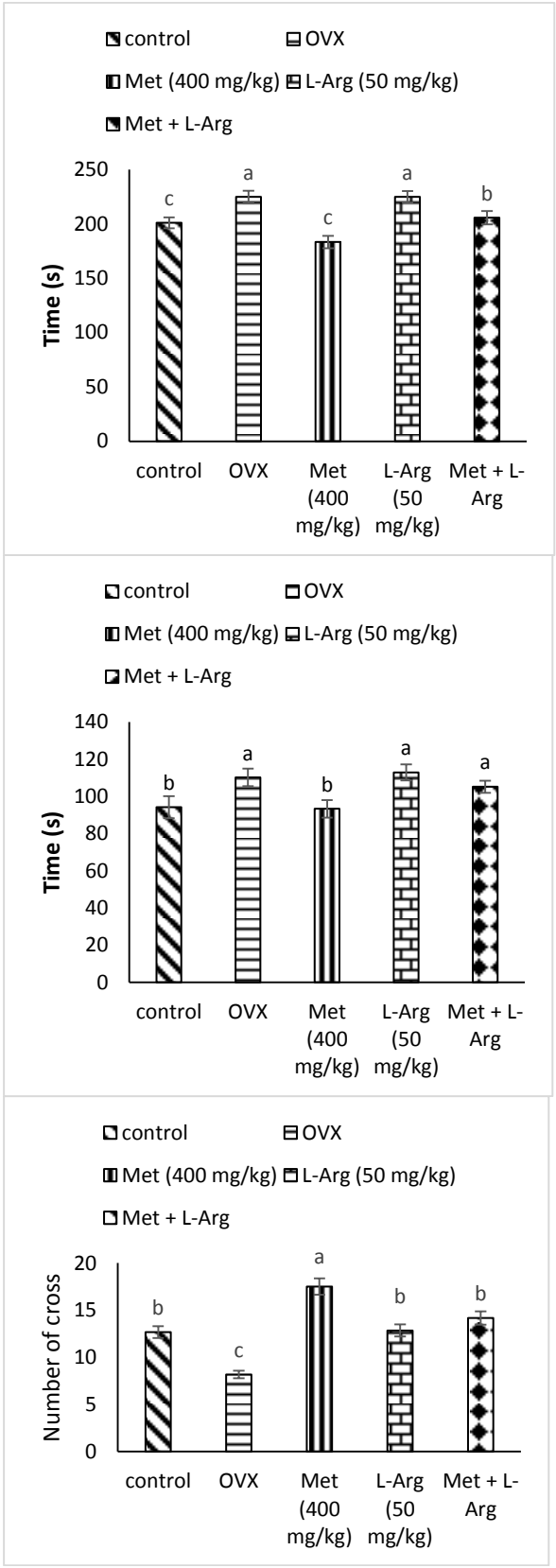
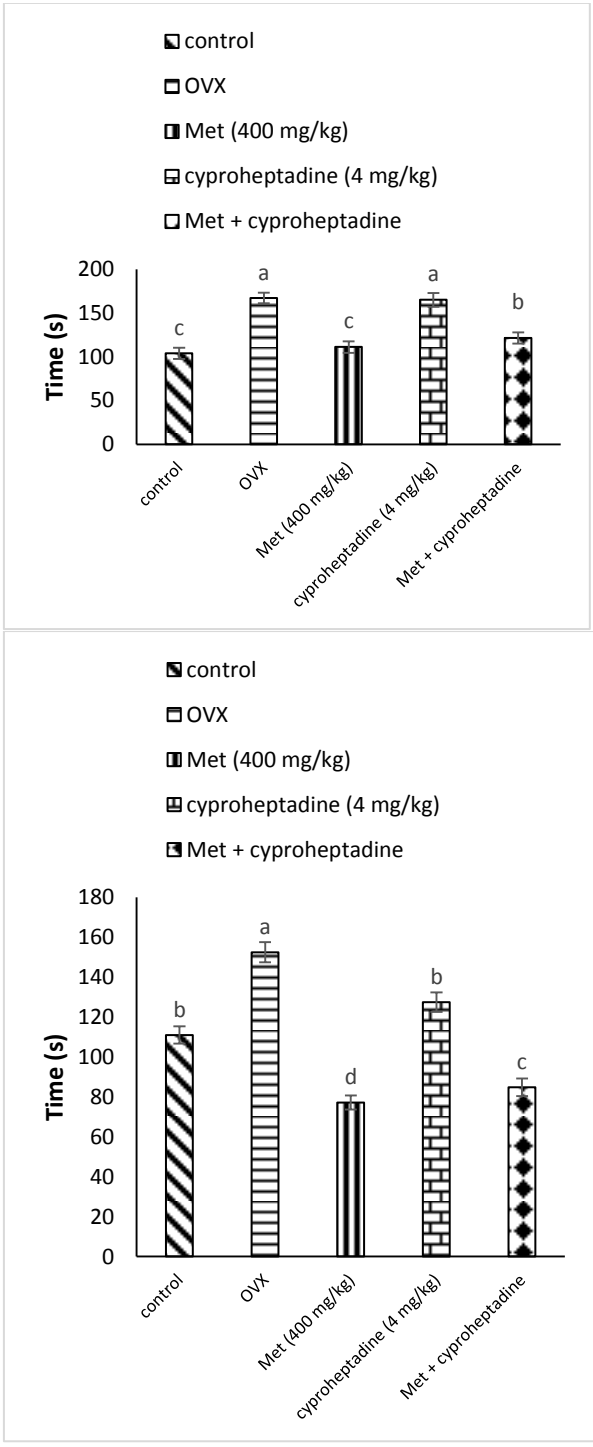


Figure 3. Effects of Metformin (400 mg/kg), L-Arg (50 mg/kg) and their co-injection FST (up), TST (middle) and OFT (down) in ovariectomized mice. Different letters (a-c) indicate significant differences between treatments (P<0.05). Met: Metformin, OVX: ovariectomy, L-Arg: L-arginine, TST: tail suspension test, FST: forced swimming test, OFT: open field (n=50).

In experiment 4, OVX increased immobility time in FST compared to the control group (P<0.05). Cyproheptadine (4 mg/kg) had no effect on immobility time (P>0.05). Co-injection of the cyproheptadine + MET increased immobility time using FST compared to MET (P<0.05) (figure 4A). Cyproheptadine + MET increased immobility time in TST compared to MET (P<0.05) (figure 4B). Also, cyproheptadine + MET increased the number of crossings in the OFT in comparison to OVX mice (P<0.05) (figure 4C).



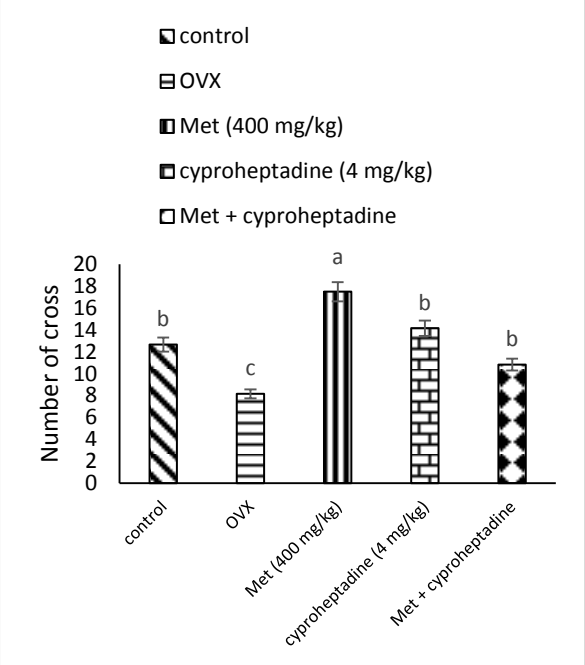
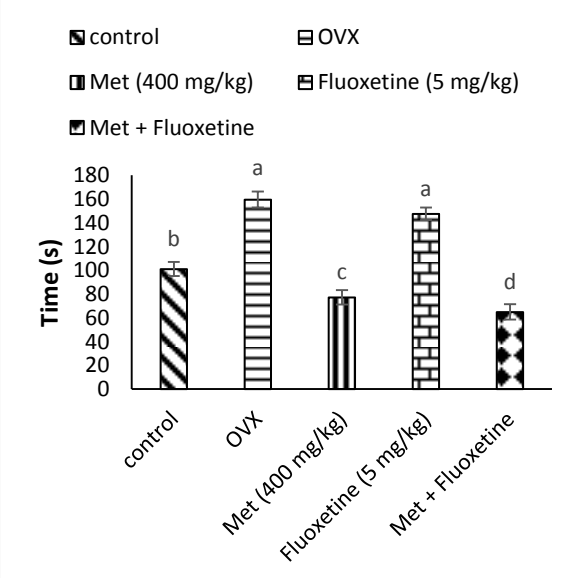


Figure 4. Effects of Metformin (400 mg/kg), Cyproheptadine (4 mg/kg) and their co-injection on FST (up), TST (middle) and OFT (down) in ovariectomized mice. Different letters (a-d) indicate significant differences between treatments (P<0.05). Met: Metformin, OVX: ovariectomy, TST: tail suspension test, FST: forced swimming test, OFT: open field (n=50).

Based on experiment 5, MET (400 mg/kg) decreased immobility time compared to the OVX-untreated rat (P<0.05). Fluoxetine (5 mg/kg) had no effect on immobility time (P>0.05). Co-injection of the fluoxetine and MET significantly decreased immobility time using FST (P<0.05) (figure 5A). Co-application of the fluoxetine and MET significantly decreased immobility time using TST (P<0.05) (figure 5B). Fluoxetine + MET significantly increased the number of crossings in the OFT in comparison to OVX mice (P<0.05) (figure 5C).



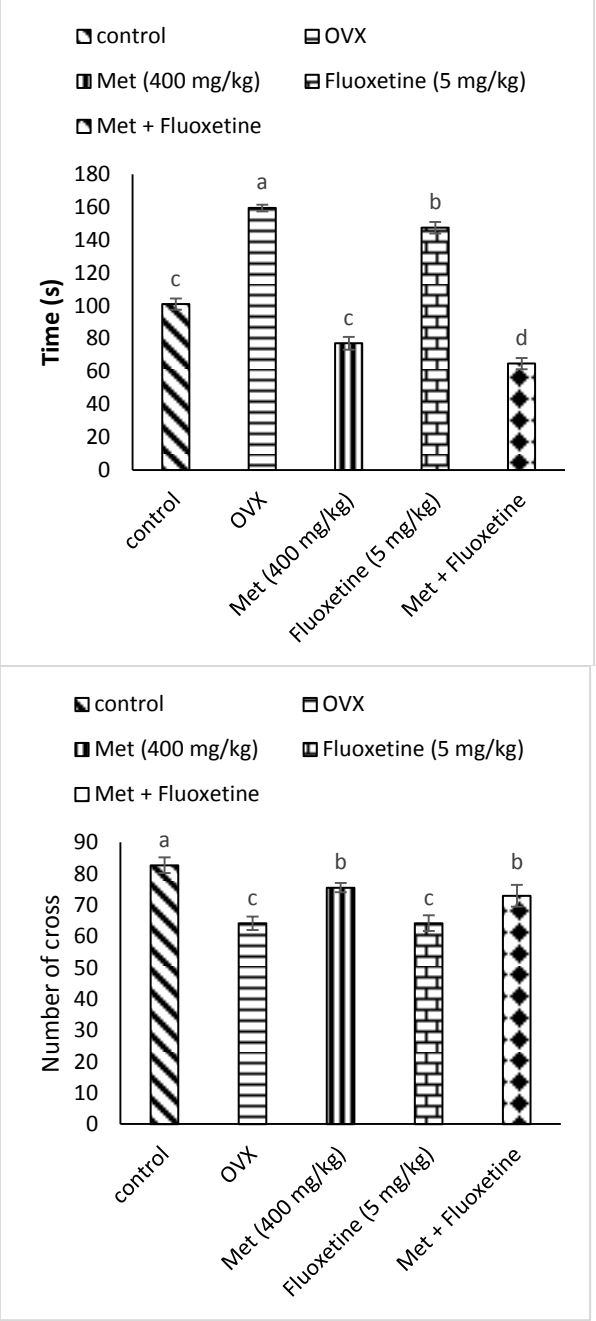


Figure 5. Effects of Metformin (400 mg/kg), Fluoxetine (5 mg/kg) and their co-injection on FST (up), TST (middle) and OFT (down) in ovariectomized mice. Different letters (a-d) indicate significant differences between treatments (P<0.05). Met: Metformin, OVX: ovariectomy, TST: tail suspension test, FST: forced swimming test, OFT: open field (n=50).

According to the table 1, OVX significantly increased MDA levels compared to the control group (P<0.05). MET (200 and 400 mg/kg) 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). MET (200 and 400 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).

Table 1. Effect of different levels Metformin on serum values of MDA, SOD, GPx and TAS in OVX mice

Group	MDA (nmol/ml)	SOD (IU/ml)	GPx (IU/ml)	TAS (nmol/ml)
Control	0.33 ± 0.04 [c]	47. 84 ± 2.13 [a]	4.24 ± 0.26 [a]	0.045 ± 0.02
Sham	0.35 ± 0.02 [c]	46.50 ± 2.11 [a]	4.35 ± 0.16 [a]	0.044 ± 0.01
OVX	1.31 ± 0.01 [a]	27.66 ± 1.11 [d]	2.23 ± 0.11 [d]	0.050 ± 0.04
Metformin (100 mg/kg)	1.3 ± 0.05 [a]	27.65 ± 1.16 [d]	2.17 ± 0.23 [d]	0.049 ± 0.02
Metformin (200 mg/kg)	0.5 ± 0.03 [c]	37.22 ± 1.21 [c]	3.42 ± 0.16 [c]	0.051 ± 0.03
Metformin (400 mg/kg)	0.8 ± 0.04 [b]	45.33 ± 1.18 [b]	3.54 ± 0.11 [b]	0.052 ±0.01

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).

4. Discussion

Depression is a serious medical illness characterized by affective disorder. However, direct mechanisms responsible for the depression is remain unclear, it seems neurotrophic factors and adult neurogenesis has prominent role in depression (22). The prevalence of depression is twice in women than men. Estrogen is well-known effects on modulating mood and emotion (23). There is a correlation with mood and estrogen level which reduction in circulating estrogen increases mood disturbances in women (24). Also, OVX-induced depression-like behavior reversed by peripheral treatment with estradiol (25). Rodents are ideal models for menopausal depressive-like state in which their strains, age at ovariectomy and time of the behavioral test following OVX influence the results (26). It is reported estradiol decreases the latency to the onset of depression in TST and TST. Perhaps estradiol related antidepressant-like actions mediates by its receptors (ERα and ERβ), serotonergic and nitrergic receptors in amygdala, hippocampus and hypothalamus (27).

As observed, MET in a dose dependent manner decreased immobility time using both TST and FST. In a similar report, Ostadhadi et al., (28) reported MET-treatment decreased immobility time after bile-duct ligation. Co-application of the L-Arginine and MET had no effect on immobility time using TST and FST tests. Co-application of the L-NAME and MET (25mg/kg) decreased MET-induced (25mg/kg) immobility time. In addition, co-injection of the L-NAME and MET decreased immobility time. Liu et al., (25) reported in rat with hyperlipidemia, injection of the MET leads to increase NO production and decreased Rho kinase activity. Metformin has antidepressant effects and improves cognitive function in depressed patients with diabetes mellitus (29). Metformin can cross the blood brain barrier and has numerous positive role as anti-inflammatory and neuroprotective function (30). Also, it is good choice for learning and memory function and cognitive impairment (31). In the central nervous system, cognition and several of the core symptoms of depression regulates in the hippocampus (32). Hippocampal neurogenesis was decreased by stress and increased by chronic antidepressant administration. Co-application of the L-NAME and MET decreased NO gene expression in OVX mice. Also, MET improves spatial learning in a water maze task (24).

It is revealed NO pathway is new treatments for numerous neuropsychiatric situations such as mood disorders (33). Nitrite is an end product of the NO metabolism which is high in depressed patients which supports its possible role in depressed patients. Several NOS inhibitors have displayed an antidepressant-like activity in the FST (26) while NOS inhibitors have antidepressant activity in the FST (34). Serum NO content enhanced in depression and the NOS inhibitors participated in recovering the mood disorder, there is proof for a dual effect of NO in the FST in mice. However, based on limitation of the current study we were not able to determine serum levels of the NO in OVX mice. Metformin increase NO and endothelial nitric oxidase synthesis (eNOS) production and regulating glucose metabolism via C-reactive protein (35). Metformin acts via AMPK phosphorylates endothelial eNOS in a calcium-independent way. Neuronal isoform of NOS is also phosphorylated by AMPK (36). NO is a gaseous signaling molecule which has been shown to play the role in some mental functions such as depression (35).

Selective serotonin reuptake inhibitors are the most commonly prescribed drugs for the treatment of depressive disorder. Serotonin has an important role

on increasing brain plasticity and enhancing susceptibility to the environment (37). Interestingly when fluoxetine administered in an enriched environment, leads to an improvement while in a stressful environment worsening depression-like endpoints such as anhedonic behavior and suppress neurogenesis (38). It is suggested co-administration of the fluoxetine and MET increase IGF2 expression in the dorsal hippocampus and effective in treatment of depression (39). In our study, MET + fluoxetine significantly amplified antidepressant activity of the MET which was in agreement to previous reports. Decrease in IGF2 hippocampal expression is associated to depression-like behavior induced through chronic restraint stress. About molecular mechanisms associated to the therapeutic action of the combined treatment of the MET + fluoxetine reported growth factor might be involved in the antidepressant action of the MET + fluoxetine treatment (37). Dorsal hippocampus is implicated in depression and is a key role for antidepressants. This area has highest expression levels of most markers of antidepressant. MET stimulates serotoninergic neurons excitability and decrease anxiety in insulin-resistant mice (40). This findings suggesting MET produces antidepressant-like activities (40). MET crosses the blood-brain barrier and perhaps via anti-inflammatory and neuroprotective properties in the central nervous system impress its anxiolytic- and antidepressant-like activity (41).

According to results, OVX increased MDA levels compared to the control group and MET reduced the MDA levels. The SOD and GPx levels diminished following OVX and MET increased the SOD and GPx levels. The positive role of the antioxidant effects of antidepressants in the treatment of major depressive disorder is well documented. Antunes et al., (42) reported antioxidant activity of the hesperidin may be mediating by O2– and modulation of the CAT and GPx activity. Following depression lipid peroxidation leads to increase serum MDA along with reduced CAT, SOD and glutathione (GSH). The imbalance between oxidative stress and antioxidative defenses leads to dysregulation of brain functions and progression of depression (43). Phospholipids in the brain are susceptible to reactive oxygen species (ROS) in the pathogenesis of depression. Depression is also accompanied by the NO which its elevated levels are linked with neuronal damage and apoptosis (44). It seems interaction between antioxidant dysfunction and NO is responsible for depressive behavior. Also, antidepressant effect of the MET mediates by increase serum serotonin, norepinephrine and decrease corticosterone and adrenocorticotropic hormone (ACTH) secretion (45). However, because of limitations of the current study we were not able to determine serum serotonin, norepinephrine, corticosterone and ACTH levels. However, more postulated mechanisms include the anti-inflammatory and antioxidant properties of metformin (46).

Conclusion

In conclusion, it seems antidepressant activity of the MET mediates by nitrergic and serotoninergic system in OVX mice.

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Conflict of interest

No potential conflict of interest was reported by the authors.

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