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# Anti-depressant effect of hesperidin in ovariectomized mice: possible involvement of dopaminergic and serotoninergic systems

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## Abstract

**Objective:** This study aimed to determine anti-depressant effect of hesperidin in ovariectomized mice and its possible interaction with dopaminergic and serotoninergic systems.

**Materials and Methods:** In experiment 1, mice were kept as control and sham groups, ovariectomized (OVX), OVX+ hesperidin (12.5 mg/kg), OVX+ hesperidin (25 mg/kg) and OVX+hesperidin (50 mg/kg). In experiment 2, mice were kept as control and sham, OVX, OVX+hesperidin (50 mg/kg), OVX+dopamine (25 mg/kg) and OVX+co-injection of hesperidin and dopamine. Experiments 3-5 were like experiment 2, except 6-OHDA (dopamine inhibitor, 100 mg/kg), fluoxetine (selective serotonin reuptake inhibitor, 5 mg/kg) and cyproheptadine (serotonergic receptor antagonist, 4 mg/kg) was injected instead of dopamine. Then, forced swimming test (FST), tail suspension test (TST) and open field test (OFT) were done. Also, serum malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (GPx) and total antioxidant status (TAS) levels were determined.

**Results:** According to the results, OVX increased immobility time in FST and TST tests as compared to control group ($P<0.05$). Hesperidin (50 mg/kg) decreased immobility time as compared to OVX group ($P<0.05$). Co-injection of hesperidin+dopamine decreased immobility time in TST and FST and increased number of crossing in OFT ($P<0.05$). Co-injection of hesperidin+6-OHDA significantly decreased antidepressant activity of the hesperidin on immobility time and decreased positive effect of the hesperidin on the number of crossing ($P<0.05$). Co-injection of hesperidin+Fluoxetine significantly amplified antidepressant activity of the hesperidin on immobility time and number of crossing ($P<0.05$). Co-injection of hesperidin+cyproheptadine decreased antidepressant activity of hesperidin on immobility time ($P<0.05$). Hesperidin (12.5, 25 and 50 mg/kg) decreased the MDA, while increased SOD and GPx levels in OVX mice ($P<0.05$).

**Conclusion:** It is assumed that antidepressant activity of hesperidin is mediated via dopaminergic and serotoninergic receptors in OVX mice.

**Keywords** Anti-depressant, Hesperidin, Serotoninergic, Dopaminergic

## 1. Introduction

Depression is one of the major prevalent mental disorders in the world. Major depressive disorder (MDD) is characterized by changes in psychosocial and physical mood as well as lack of interest in the surroundings and eventually leads to suicide (1). The MDD is a complex impairment which alters brain physiological function, emotional and cognitive processes and its incidence rate is higher in women than men because of hormonal fluctuations (2). Ovarian hormones are known as important regulators of emotional status, mood regulation, and cognition. Changes in estradiol levels during the menstrual cycle, parturition and menopause enhance depression risk and mood disruption (3). Since role of the ovarian estradiol on

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MDD has been identified, numerous researches have been done to investigate the possible neurological mechanisms. Bilateral OVX mice show depressive-like behavior (4) and estradiol therapy showed antidepressant-like effect in OVX mice (5). Although the direct mechanism for effects of the estradiol in MDD is not fully elicited, it acts by modulation of serotonergic and dopaminergic (DAergic) systems (5, 6).

Several neurotransmitters in the hippocampus interplay with MDD (7). Dopamine (DA) is the main catecholamine neurotransmitter which is expressed in several nuclei of the CNS such as substantia nigra, ventral tegmental area, and hypothalamus which play key roles in emotion, locomotor activity, cognition, and appetite regulation (8). There are evidences for the mediatory role of the DAergic system in depression. For instance, concentration of the homovanillic acid, a chief metabolite of the DA, is low in cerebrospinal fluid and plasma of the MDD patients. Also, decreased DA levels and its receptors leads to mood impairment and Parkinson's disease (9). Administration of the L-DOPA is beneficial for treatment-resistant depression patients (10).

Serotonin is the main neurotransmitter for mood and behavior regulation and its deficiency leads to depression (11). There is a neurological interaction between serotonin and ovarian hormones in which estrogen and progesterone are able to modulate serotonergic function as an underlying factor for depression (12). The ovarian hormones act on raphe nuclei and regulate serotonergic function. Estrogen increases serotonin synthesis and/or decreases serotonin reuptake which facilitates serotonergic transmission and leads to alleviating depressive symptoms (13). Antidepressant drugs such as selective serotonin reuptake inhibitors (SSRIs) and tricyclic anti-depressants affecting via monoaminergic system can be used for pathogenesis of the depression (14). Side effects such as sedation, blurred vision, constipation, seizures and sexual dysfunction are major limitations of these drugs (14). Thus, there is a growing interest for new bioactive antidepressant drugs with fewer side effects.

Hesperidin is the major flavonoid isolated from citrus fruits (15). The hesperidin molecule is composed of aglycone unit known as hesperetin and a disaccharide, rutinose (16). Hesperidin has several biological effects such as antioxidant, anti-inflammatory, antimicrobial, anti-carcinogenic and anti-allergic effects (15). Neuropharmacological properties have been reported for the hesperidin (17). It has a high potential for radical scavenging and protective effects and can cross blood brain barrier (18). Hesperidin promotes neuronal survival, differentiation and neuroprotective capacity of the astrocytes (19). It is assumed that flavonoid compounds of the hesperidin have neuroprotective activity in Parkinson's disease patients (PPD) (20). The antidepressant-like effect of the hesperidin has been reported in forced swimming test (FST) and tail suspension test (TST) tests (21). Hesperidin (1 mg/kg) decreases immobility time in FST in mice (22). Hesperidin has beneficial effect on memory impairment by DA and antioxidant enhancement which at a dose of 50 mg/kg can improve depressive-like behavior in TST (20). Moreover, hesperidin suppresses depressive-like behaviors induced by intra-striatal injection of the 6-hydroxydopamine (6-OHDA) in Parkinson's disease (20). However, there is limited information about antidepressant effect of hesperidin in OVX mice. So, the main purpose of the current paper was to determine the antidepressant effect of hesperidin in OVX mice and its possible interaction with DAergic and serotoninergic systems.

## 2. Materials and Methods

### 2.1. Animals

A total of 240 adult female NMRI mice were supplied from the Pasteur Institute (Tehran, Iran) and kept five mice/cage in standard plastic cages at laboratory conditions (temperature of 22 ± 2°C and 12/h light/dark cycle) with ad libitum access to standard chow pellet (Pars Dam Co, Tehran, Iran) and fresh water. Animal handling and experimental procedures were performed 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) (23).

### 2.2. Drugs

Hesperidin, DA, 6-OHDA (dopamine inhibitor), cyproheptadine (serotonergic receptor antagonist) and fluoxetine (selective serotonin reuptake inhibitor) were obtained from the SigmaAldrich (USA). Hesperidin was dissolved by the sequential addition of dimethylsulfoxide (DMSO) up to a final concentration of 5%, a water solution of 0.25% Tween 80 up to a final concentration of 20% and saline to complete 100% volume (24).

### 3.3. Experimental procedure for ovariectomy

All surgical procedures were performed under anesthesia by intraperitoneal (i.p.) injection of ketamine hydrochloride (50 mg/kg) and xylazine hydrochloride (5 mg/kg) (Alfasan, Woerden, Holland). Following anesthesia, the lumbar dorsum was shaved and exposed skin was prepared for aseptic surgery (a 10% povidene-iodine scrub followed by a sterile saline wipe). A 1-2 cm incision was done in the midline on the lumbar vertebral line. Approximately 1 cm to each flank, paraovarian fatty tissue was identified and pulled out through the incision. The

exposed ovary and associated oviduct were removed. Then, skin incision was sutured. In the sham group, the paraovarian fatty tissues and ovaries were just retracted and replaced (25, 26). All behavioral tests were done 10 days after recovery (5).

## 2.4. 17β-estradiol assay

Blood samples were taken from each mouse via cardiac puncture for 17β-estradiol (E2) assay by direct and competitive chemiluminescence immunoassay (CLIA) detection using LIASION Estradiol (310400) kit and according to manufacturer's instructions (DiaSorin Inc., USA) [27]. Serum E2 level lower than 20±2 pg/ml was used for the accuracy of the OVX (5).

## 2.5. Experimental procedure

OVX mice were randomly allocated into 5 experimental groups (n=48 in each experiment and 8 mice in each group). In experiment 1, control group was without surgery and injected with saline (10 ml/kg) at 1 h prior to the test; sham group had no ovariectomy (OVX) and injected with saline (10 ml/kg) at 1 h prior to the test; in OVX group, mice were i.p injected with saline (10 ml/kg) at 1 h prior to the test; in + hesperidin (12.5 mg/kg) group, mice were i.p injected with hesperidin 1 h prior to the test; in OVX + hesperidin (25 mg/kg) group, mice were i.p injected with hesperidin 1 h prior to the test; in OVX + hesperidin (50 mg/kg) group, mice were i.p injected with hesperidin 1 h prior to the test. Then, 1 hour later, FST, TST and OFT were done. In experiment 2, control group without surgery and injected with saline (10 ml/kg) at 1 h prior to the test; sham group had no OVX and injected with saline (10 ml/kg) at 1 h prior to the test; in OVX group, mice were i.p injected with saline (10 ml/kg) at 1 h prior to the test; in OVX + hesperidin (50 mg/kg) group, mice were i.p injected with hesperidin 1 h prior to the test; in OVX + DA group, mice were i.p injected with DA (25 mg/kg) at 1 h prior to the test and 1 hour later, FST, TST and OFT were done; in OVX + hesperidin + DA, mice were i.p injected with hesperidin (50 mg/kg) at 1 h prior to the test, after 15 minutes were i.p injected with DA (25 mg/kg) and 45 minutes later, tests were done. In experiment 3, control group without surgery and injected with saline (10 ml/kg) at 1 h prior to the test; sham group had no OVX and were injected with saline (10 ml/kg) at 1 h prior to the test; in OVX group, mice were i.p injected with saline (10 ml/kg) at 1 h prior to the test; in OVX + hesperidin (50 mg/kg) group, mice were i.p injected with hesperidin 1 h prior to the test; in OVX + 6-OHDA group, mice were i.p injected with 6-OHDA (10 mg/kg) at 1 h prior to the test and 1 hour later, FST, TST and OFT were done; in OVX + hesperidin + 6-OHDA group, mice were i.p injected with hesperidin (50 mg/kg) at 1 h prior to the test, after 15 minutes, they were i.p injected with 6-OHDA (10 mg/kg) and 45 minutes later, tests were done. In experiment 4, control group without surgery and injected with saline (10 ml/kg) at 1 h prior to the test; sham group had no OVX and injected with saline (10 ml/kg) at 1 h prior to the test; in OVX group, mice were i.p injected with saline (10 ml/kg) at 1 h prior to the test; in OVX + hesperidin (50 mg/kg) group, mice were i.p injected with hesperidin 1 h prior to the test; in OVX + fluoxetine (5mg/kg) at 1 h prior to the test and 1 hour later, FST, TST and OFT were done; in OVX + hesperidin + fluoxetine group, mice were i.p injected with hesperidin (50 mg/kg), after 15 minutes, they were i.p injected with fluoxetine (5 mg/kg) and 45 minutes later, tests were done. In experiment 5, control group without surgery and injected with saline (10 ml/kg) at 1 h prior to the test; sham group had no OVX and injected with saline (10 ml/kg) at 1 h prior to the test; in OVX group, mice were i.p injected with saline (10 ml/kg) at 1 h prior to the test; in OVX + hesperidin (50 mg/kg) group, mice were i.p injected with hesperidin 1 h prior to the test; in OVX + cyproheptadine group, mice were i.p injected with cyproheptadine (4 mg/kg) at 1 h prior to the test and 1 hour later, FST, TST and OFT were done; in OVX + hesperidin + cyproheptadine, mice were i.p injected with hesperidin (50 mg/kg) at 1 h prior to the test, after 15 minutes, they were i.p injected with cyproheptadine (4 mg/kg) and 45 minutes later, tests were done. At the end of each experiment, blood samples were taken and serum MDA, SOD, GPx and TAS levels were determined.

## 2.6. Forced swimming test (FST)

FST was carried out following the protocol as described previously in mice (28). Each mouse was plunged into a glass cylinder (height: 25 cm; diameter: 15 cm) containing 10 cm of water (25 ± 1 °C) for 15 min (pre-test session). 24 h later, the mouse was placed in the cylinder again and left for 6 min period (test session). The immobility time for the mouse was described when it ceased struggling and remained floating motionless in the water, making only small movements necessary to keep its head above water. The total duration of immobility during the last 4 min of the 6 min testing period was measured.

## 2.7. Tail suspension test (TST)

TST is one of the commonest techniques for assessing antidepressant-like activity in mice [29]. The TST was done based on the method stated by Steru et al. (30). Briefly, the mice were away from the nearest objects and were both acoustically and visually isolated from observing or interacting with each other. Then, suspended 50 cm above the floor by adhesive tape placed approximately 1 cm from the extremity of the tail, in such a position that it cannot escape or hold on to nearby surfaces. Immobility time was recorded for 6 minutes. Mice were considered immobile only when they had no strong body shaking and movement of the

limbs as they hanged passively and completely motionless.

## 2.8. Open field test (OFT)

OFT was used to determine possible effects of hesperidin on the locomotor and exploratory activities. The open field test was done using a 45×45×30 cage. The flour of open field cage was divided by masking tape markers into 3×3 squares. Each animal was placed individually at the center of the apparatus and observed for 6 min to record the locomotion (number of segments crossed with four paws) (24).

## 2.9. Antioxidant activity

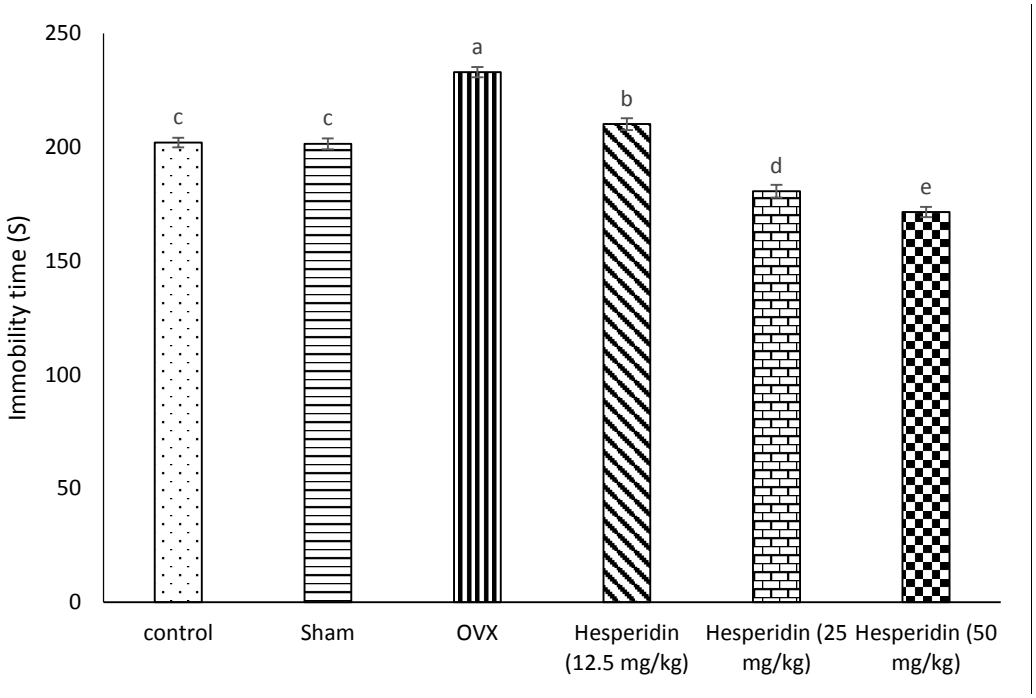
At the end of the tests, blood samples were collected via cardiac puncture and serum malondialdehyde (MDA), SOD, GPx and total antioxidant capacity (TAC) were determined using ZellBio GmbH (Germany) assay kits (ZB-MDA-48A, ZB-SOD-A48, ZB-GPX-A48 and ZB-TAC-48A, respectively).

## 2.10. Statistical analysis

Data were analyzed by one-way analysis of variance (ANOVA) and is presented as the mean ± SEM. For treatments found to have an effect according to the ANOVA, mean values were compared with Tukey's test. $P<0.05$ was considered to indicate significant differences between the treatments.

## 3. Results

The results of the anti-depressant and antioxidant effect of hesperidin in ovariectomized mice are presented in figures 1-5 and table 1. As seen in figure 1, no significant difference observed on immobility time in FST and TST in sham compared to control group ($P>0.05$). The OVX significantly increased immobility time in FST and TST compared to control group ($P<0.05$). Hesperidin in a dose dependent manner decreased depression induced immobility time in comparison to OVX group ($P<0.05$). There was no significant difference in the number of crossing in OFT between sham and control mice ($P>0.05$). The OVX significantly decreased number of crossing in OFT compared to the control group ($P<0.05$). Hesperidin (12.5 mg/kg) had no effect on OFT ($P>0.05$) while 25 and 50 mg/kg of hesperidin significantly increased the number of crossing in OF test in comparison to OVX mice ($P<0.05$).



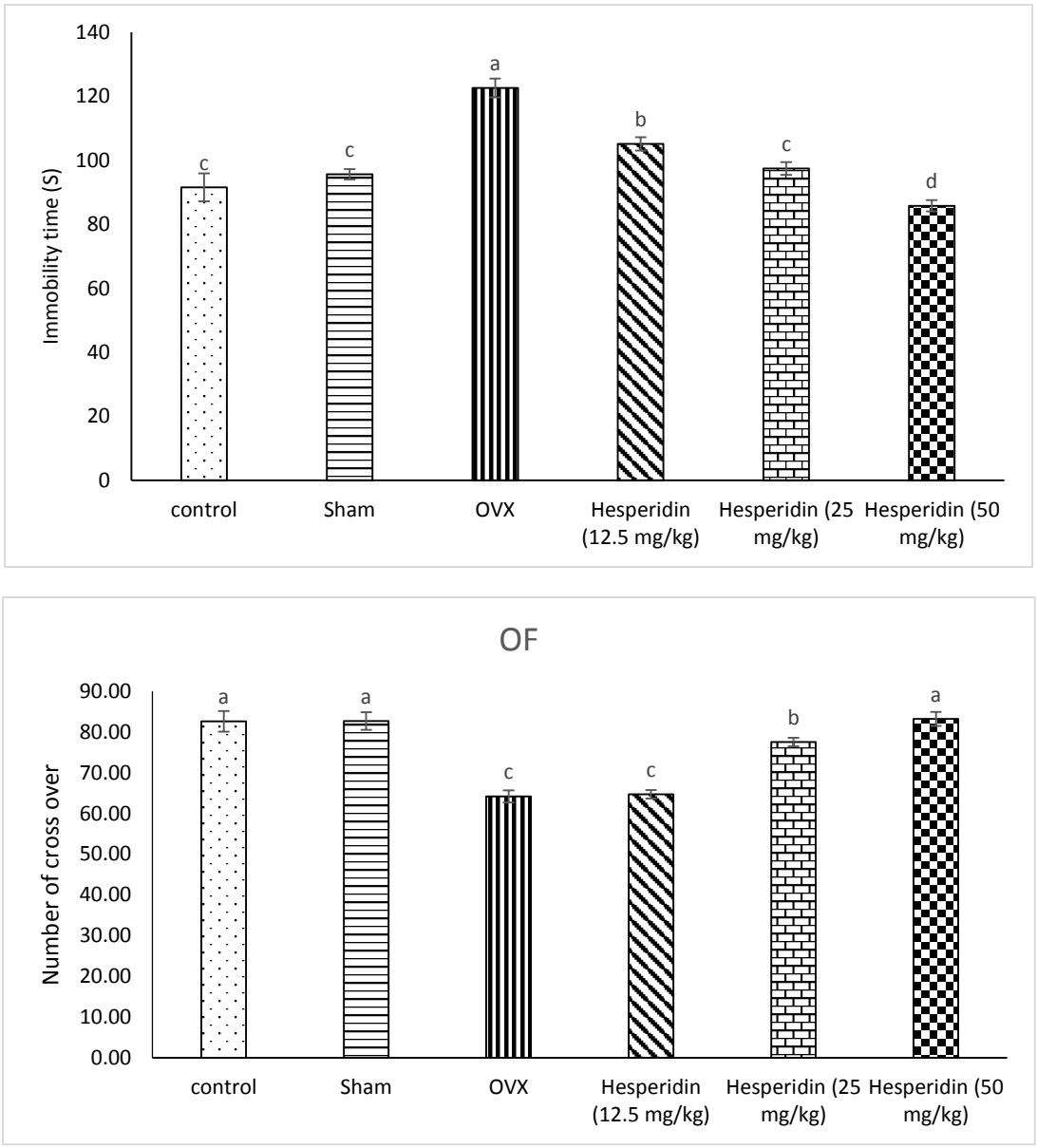



**Figure 1.** Effects of hesperidin (12.5, 25 and 50 mg/kg) on FST (up), TST (middle) and OFT (down) tests in ovariectomized mice. Different letters (a-c) indicate significant differences between treatments (P<0.05). TST: tail suspension test, FST: forced swimming test, OF: open field.

According to the figure 2, no significant difference was observed for immobility time in FST and TST in control and sham groups (P>0.05). The OVX significantly increased immobility time in FST and TST as compared to control mice (P<0.05). Hesperidin (50 mg/kg) significantly reduced immobility time in comparison with OVX group (P<0.05). The dopamine (25 mg/kg) had no effect on immobility time as compared to OVX mice (P>0.05). Co-injection of the hesperidin (50 mg/kg) + dopamine (25 mg/kg) significantly decreased immobility time in comparison with the control group (P<0.05). The OVX significantly diminished number of crossing in OFT as compared to OVX mice (P<0.05). Hesperidin (50 mg/kg) significantly increased number of crossing in OFT in comparison with OVX group (P<0.05). The dopamine (25 mg/kg) had no effect on OFT compared with OVX group (P>0.05). Co-injection of the hesperidin (50 mg/kg) + dopamine (25 mg/kg) significantly amplified number of crossing in comparison with OVX mice (P<0.05).

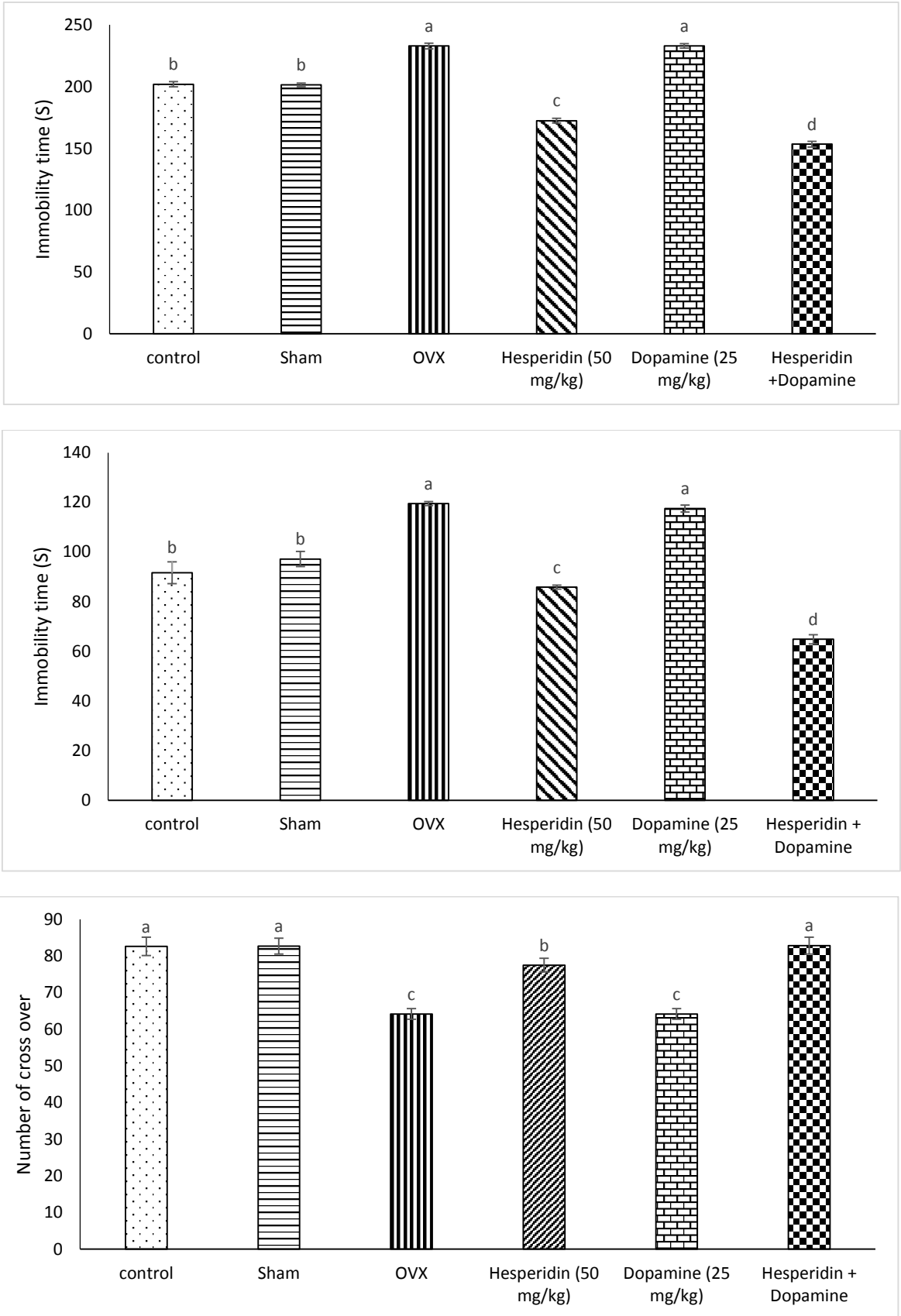
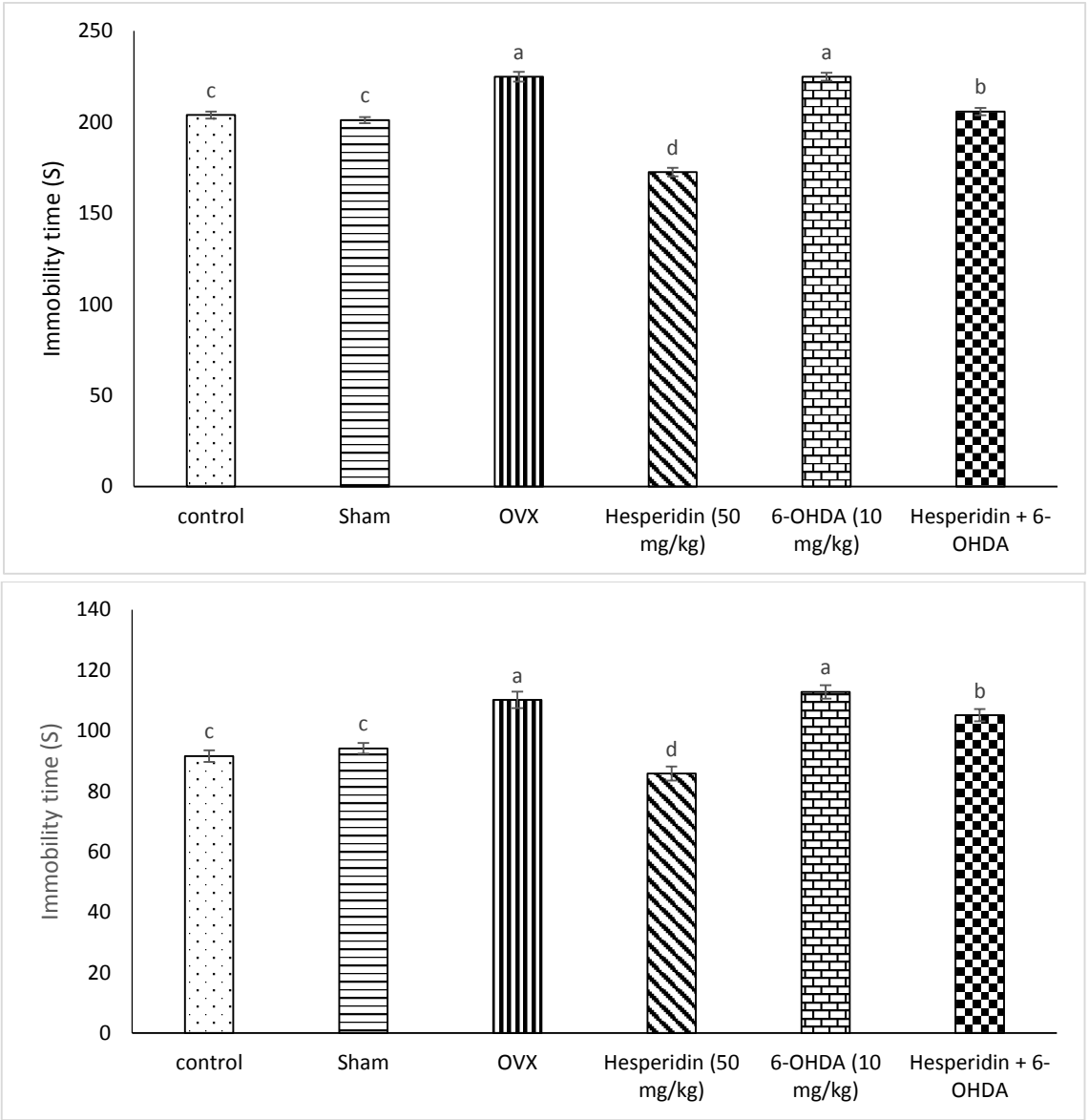



**Figure 2.** The effects of hesperidin (50 mg/kg), dopamine (50 mg/kg) and their co-injection on FST (up), TST (middle) and OFT (down) tests in in ovariectomized mice. Different letters (a-c) indicate significant differences between treatments ($P<0.05$). TST: tail suspension test, FST: forced swimming test, OF: open field.

Based on the results, immobility time significantly increased in OVX group as compared to control group (P<0.05). Hesperidin (50 mg/kg) significantly lessened immobility time in comparison with OVX mice (P<0.05). 6-OHDA (10 mg/kg) had no effect on immobility time as compared to OVX mice (P>0.05). Co-injection of the hesperidin (50 mg/kg) + 6-OHDA (10 mg/kg) significantly decreased the antidepressant activity of the hesperidin on immobility time as compared to OVX group (P<0.05). The OVX significantly reduced the number of crossing compared to OVX mice (P<0.05). The hesperidin (50 mg/kg) significantly increased number of crossing comparison to OVX mice (P<0.05). Co-injection of the hesperidin (50 mg/kg) + 6-OHDA (10 mg/kg) decreased the positive effect of the hesperidin on number of crossing in comparison with OVX group (P<0.05). It seems that the antidepressant activity of the hesperidin is mediated via dopaminergic system in ovariectomized mice (figure 3).



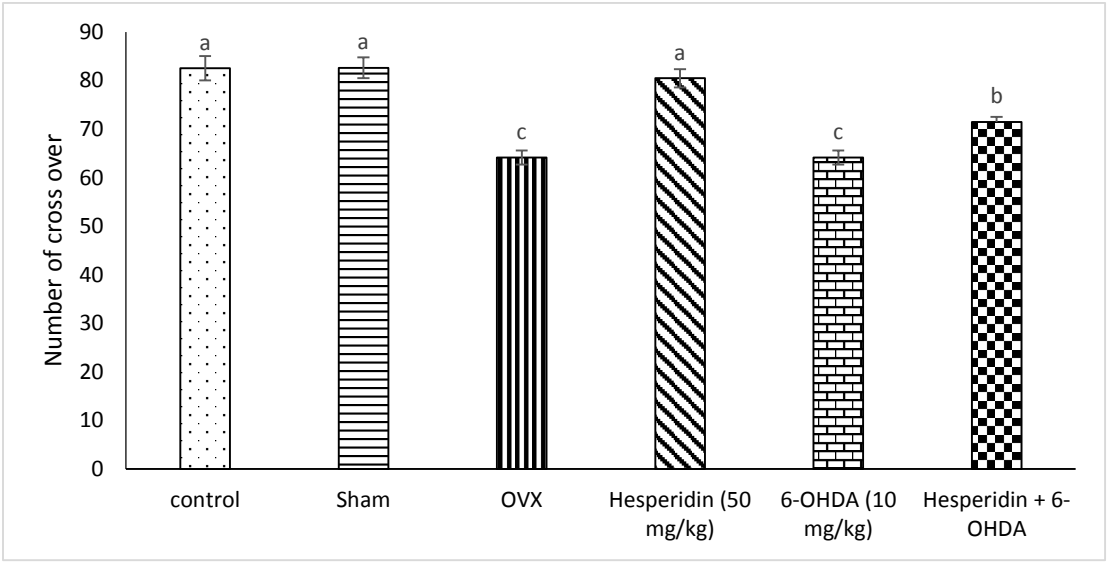
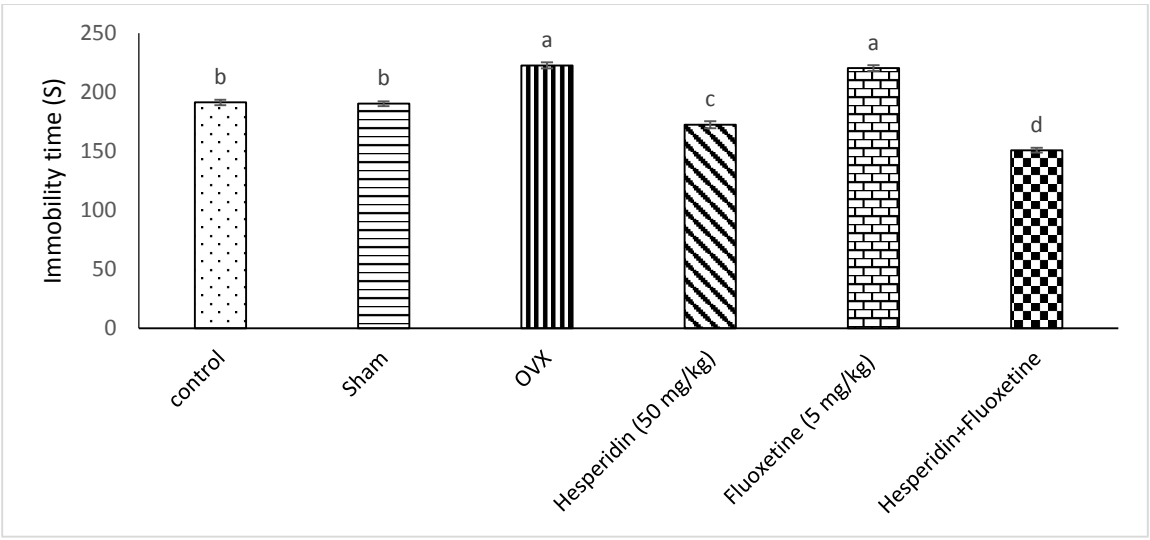



**Figure 3.** The effects of hesperidin (50 mg/kg), 6-OHDA (10 mg/kg) and their co-injection on FST (up), TST (middle) and OFT (down) tests in in ovariectomized mice. Different letters (a-d) indicate significant differences between treatments (P<0.05). OHDA: dopamine inhibitor, TST: tail suspension test, FST: forced swimming test, OF: open field.

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



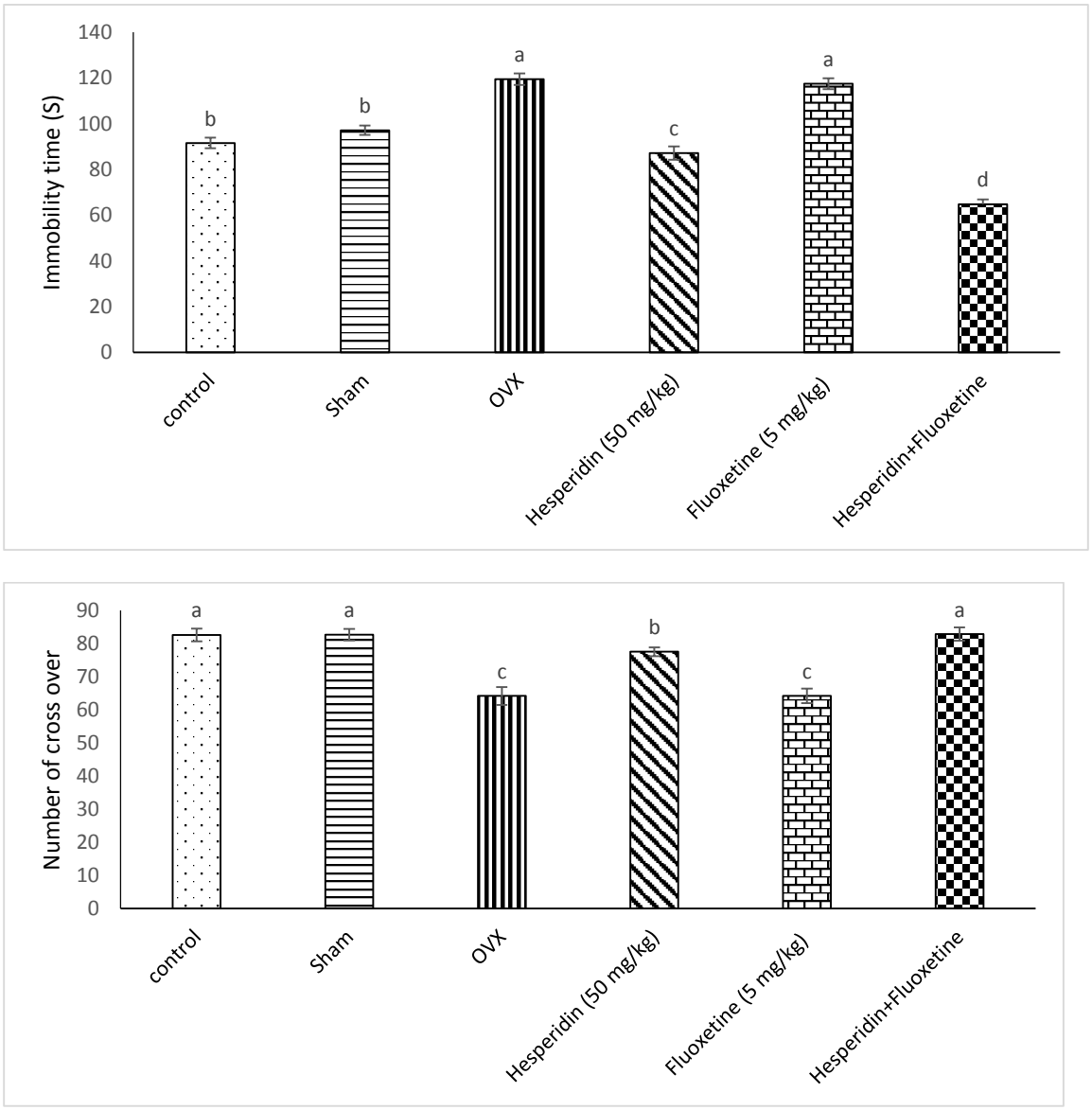



**Figure 4.** The effects of hesperidin (50 mg/kg), fluoxetine (5 mg/kg) and their co-injection on FST (up), TST (middle) and OFT (down) tests in in ovariectomized mice. Different letters (a-d) indicate significant differences between treatments (P<0.05). TST: tail suspension test, FST: forced swimming test, OF: open field.

As observed in figure 5, OVX significantly increased immobility time in FST and TST as compared to the control group (P<0.05). Hesperidin (50 mg/kg) significantly reduced depression-induced immobility time in comparison with OVX mice (P<0.05). The cyproheptadine (4 mg/kg) had no effect on immobility time as compared to OVX group (P>0.05). Co-injection of the hesperidin (50 mg/kg) + cyproheptadine (4 mg/kg) significantly decreased antidepressant activity of the hesperidin on immobility time as compared to OVX group (P<0.05). The OVX significantly reduced number of crossing as compared to OVX mice (P<0.05). The 50 mg/kg of the hesperidin significantly increased number of crossing in comparison with OVX group (P<0.05). Co-injection of the hesperidin (50 mg/kg) + cyproheptadine (4 mg/kg) increased number of crossing in comparison with OVX mice (P<0.05). Perhaps, the antidepressant activity of the hesperidin is mediated via the serotoninergic system in ovariectomized mice.

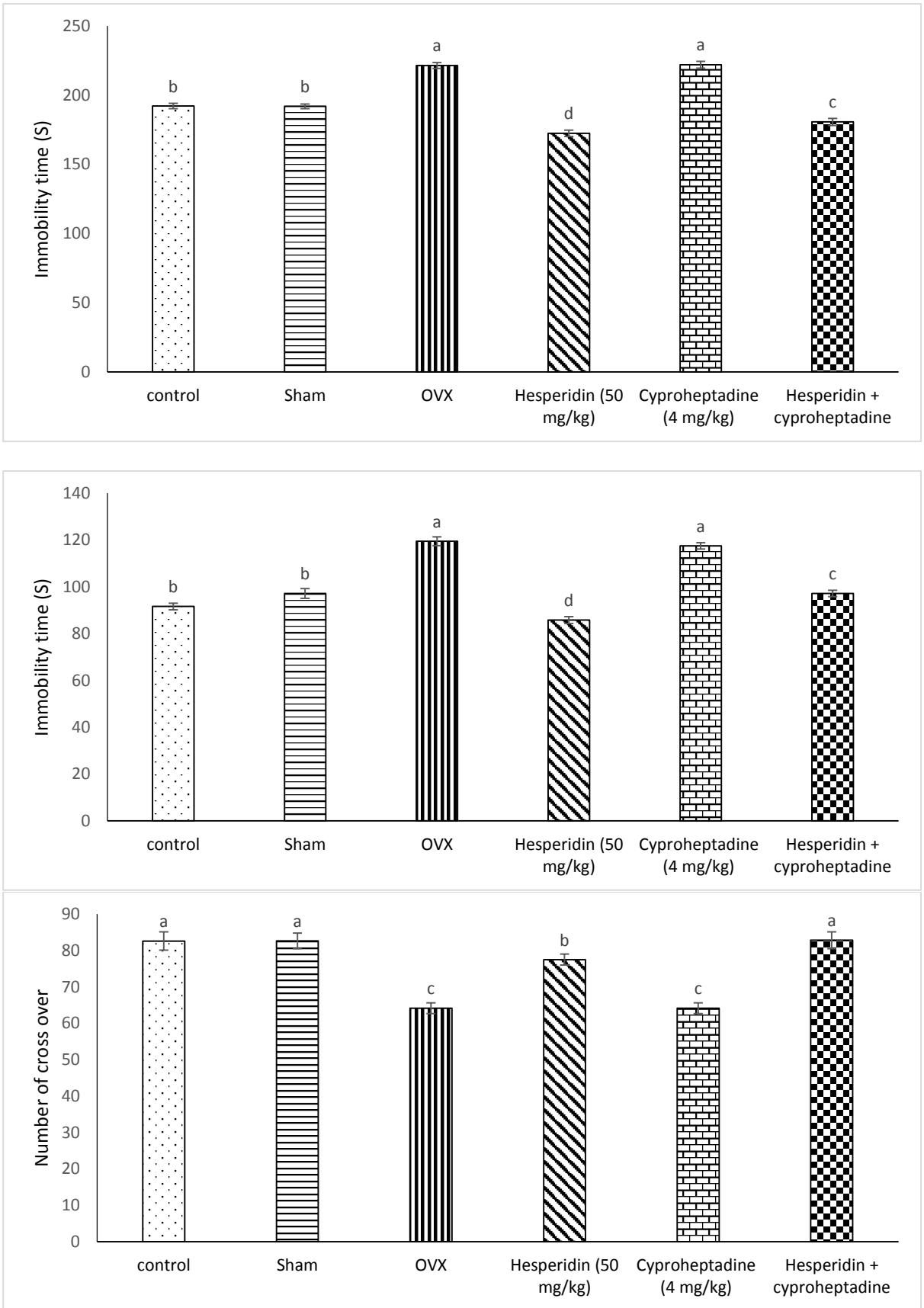



**Figure 5.** The effects of hesperidin (50 mg/kg), cyproheptadine (4 mg/kg) and their co-injection on FST (up), TST (middle) and OFT (down) tests in in ovariectomized mice. Different letters (a-d) indicate significant differences between treatments ($P<0.05$). TST: tail suspension test, FST: forced swimming test, OF: open field.

Effect of different doses of hesperidin on serum values of MDA, SOD, GPx and TAS in ovariectomized mice is presented in table 1. As seen, OVX significantly increased MDA level as compared to the control group (P<0.05). Hesperidin (12.5, 25 and 50 mg/kg) significantly decreased MDA level as compared to the OVX mice (P<0.05). SOD and GPx levels significantly diminished following OVX (P<0.05). Hesperidin (12.5, 25, and 50 mg/kg) significantly elevated SOD and GPx levels in comparison with OVX mice (P<0.05). However, no significant fluctuation on TAS was observed (P>0.05).

**Table 1.** Effect of different doses of hesperidin on serum values of malondialdehyde, superoxide dismutase, glutathione peroxidase and total antioxidant status in ovariectomized mice

| *Group* | **MDA (nmol/ml)** | **SOD (IU/ml)** | **GPx (IU/ml)** | **TAS (nmol/ml)** |
|---|---|---|---|---|
| **Control** | 5.86 ± 0.11 [c] | 65.84 ± 2.34 [a] | 6.32 ± 0.40 [a] | 1.83 ± 0.05 |
| **Sham** | 6.01 ± 0.17 [c] | 53.50 ± 2.20 [a] | 6.29 ± 0.51 [a] | 1.82 ± 0.01 |
| **OVX** | 12.31 ± 0.31 [a] | 17.66 ± 1.11 [d] | 2.17 ± 0.43 [d] | 1.80 ± 0.04 |
| **Hesperidin (12.5 mg/kg)** | 11.58 ± 0.54 [a] | 17.66 ± 1.16 [d] | 2.21 ± 0.24 [d] | 1.83 ± 0.03 |
| **Hesperidin (25 mg/kg)** | 8.46 ± 0.22 [b] | 31.66 ± 1.21 [c] | 3.33 ± 0.11 [c] | 1.83 ± 0.02 |
| **Hesperidin (50 mg/kg)** | 6.53 ± 0.31 [c] | 51.53 ± 1.18 [b] | 4.45 ± 0.20 [b] | 1.82 ±0.02 |

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

The gonadal E2 has key role in emotion and mood, depression and cognitive behavior during the reproductive life stage. In women with MDD vulnerability, low E2 level increases risk for depressive episodes and E2 may support the healthy functioning of amygdala, hippocampus, and hypothalamus (3). Rodents are ideal models for sex hormone-related depressive-like state in which their strains, age at ovariectomy and time of the behavioral test following OVX influence the results (5). In the current study, the serum E2 levels lower than 20 ± 2 pg/ml in adult female NMRI mice were used as OVX which was similar to previous reports (5). It is reported that E2 decreases the latency to the onset of depression in TST and TST. Even though the direct mechanism for antidepressant-like actions of the E2 is not well-understood, its effect is mediated by its receptors (ERα and ERβ), serotonergic and DAergic receptors in amygdala, hippocampus, and hypothalamus (31).

Alterations in the DAergic system suggest for role of the DA in depressive-like behaviors in Parkinson's disease-related depression. It is reported that daily hesperidin (50 mg/kg) for 28 d may attenuate 6-OHDA-induced catecholamine neurotoxicity, thereby maintaining the concentration of DA and its levels of metabolites at normal or close to normal levels. The activities of the DAergic neurons can determine DA and homovanilic acid levels in the brain or in the cerebrospinal fluid (32). In the current study, interaction was observed between hesperidin and DAergic system which was in agreement with previous reports. However, because of the limitations of the current study, we were not able to determine DA and its levels of metabolites following administration of the hesperidin in OVX mice. Most antidepressants act by increasing serotonin and norepinephrine levels in hippocampus, limbic, thalamic, and prefrontal cortical area of depressed patients (33). It is reported that anti-nociceptive activity of the hesperidin is mediated by opioidergic and serotonergic systems (34). The serotonergic and DAergic systems may have major roles for antidepressant activity of the hesperidin and oral administration of 400 mg/kg of hydroalcoholic extracts (their main flavonoids including rutin and hesperidin) increases serotonin and dopamine levels in the brain (35). For instance, it is reported that administration of the hesperidin (1 mg/kg for 14 days) decreases immobility time and increases DA and serotonin levels in hippocampus and cerebral cortex in mice (36). The involvement of serotonin in the effects of the hesperidin in central and peripheral nervous system have been reported. Souza et al. (21) reported hesperidin increases serotonin but not DA levels in mice brain, which may be due to modulation of 5HT-1A receptor.

In the current study, interaction observed between hesperidin and DAergic system was in agreement with previous reports. Hesperidin decreases 5-HT-induced delayed emptying of the stomach, being similar to selective 5-TH3 receptor antagonist, ondansetron (37). Hesperidin has antagonistic effects on 5-HT2B receptors (38). Also, hesperidin has antiplatelet effects through inhibition of arachidonic acid-induced serotonin secretion (39). Based on evidences,

interconnection exists between DAergic and serotoninergic system and intracerebroventricular injection of the L-DOPA attenuates the effects of fluoxetine acting at serotonergic terminal level. It is assumed that endogenous serotonin is replaced by L-DOPA-derived DA, which can act as false neurotransmitter and reduce the effect of serotonin (9). Co-injection of the fluoxetine and L-DOPA leads to competition for the mechanisms involved in clearing or storing DA and serotonin. The antidepressant-like effect of hesperidin on mice depends on its interaction with 5-HT1A receptors which significantly increases serotonin levels but not DA in brain (21). Fluoxetine through activation of 5-HT1A improves 6-OHDA-induced catalepsy in L-DOPA-treated rats. In serotonergic neurons, DA is co-stored with serotonin in the same vesicles and is known as a false transmitter (40). So, these findings support the role of interaction of the DAergic and serotoninergic system for antidepressant-like effect of hesperidin. However, because of limitations of the current work, we were not able to determine DA and serotonin levels in the CNS of the hesperidin-treated OVX mice.

Antidepressant-like activity of the hesperidin is mediated via opioid and 5HT1A receptors as well as l-arginine-NO-cGMP pathway. It is also reported to increase brain-derived neurotrophic factor (BDNF) levels in the brain (24). In this regard, recently, Khodadaeh et al. (2020) reported that hesperidin (0.5 and 1 mg/kg) injection on days of 5, 8, 11, 14 and 17 of pregnancy decreases immobility time in TST and FST on postpartum mice. Hesperidin (0.01, 0.3, and 1 mg/kg) has antidepressant-like effect in TST and decreases nitrate/nitrite as well as increased hippocampal BDNF in the hippocampus of mice (24). Based on literature, NO/cGMP pathway has key role in antidepressant effect of hesperidin (24). It is reported that nitrate/nitrite levels decrease in the hippocampus of hesperidin-treated mice. Anti-depressant activity of the hesperidin is inhibited by pretreatment with L-arginine (precursor of nitric oxide). Also, administration of the hesperidin increases BDNF level in the hippocampus of mice (24). Perhaps, antidepressant-like activity of the flavonoids is mediated via BDNF (17). Besides, flavonoids exhibit a wide range of pharmacological activities such as sedative effect, however, hesperidin (50 mg/kg) had no sedative or excitatory effects on the animals [20] and in the current study no sedative activity was observed in OVX mice.

Hesperidin attenuates 6-OHDA-induced reduction in GPx and CAT activity, total reactive antioxidant potential and declines DA levels in the striatum (20). Administration of the hesperidin (0.5 and 1 mg/kg) during the pregnancy decreases MDA levels on postpartum. Also, pre-partum administration of hesperidin (0.1, 0.5, and 1 mg/kg) significantly increases SOD and GPx levels in postpartum mice (41). Hesperidin can enhance glutathione, SOD, CAT and decrease MDA and nitrite level (42). Also, injection of the 20, 40 and 80 mg/kg of hesperidin reverses the levels of serum hepatic CAT, SOD, GPx and GST enzyme levels (43) which our result was similar to these reports. Antioxidant activity of hesperidin is mediated by scavenging activity. Hesperidin has protective effect against reactive oxygen species (ROS) production and oxidative stress. Hesperidin increases antioxidant enzymes CAT, SOD and glutathione S-transferase (GST) level (44). The CAT, SOD, GPx, and GST play important roles on ROS. Hesperidin has protective effect against oxidative damage due to the ability of increase antioxidant activity. There is a correlation between depressive disorders and increased oxidative stress, neuro-inflammation and diminished anti-oxidant defenses (45). The positive role of the antioxidant effects of antidepressants in the treatment of major depressive disorder is well documented. Antunes et al. (20) reported that antioxidant activity of the hesperidin may be mediated by O2– and modulation of the CAT and GPx activity. Previous reports revealed hesperidin prevents cell membrane damage in neurodegenerative diseases (46).

In conclusion, these results revealed antidepressant activity of hesperidin which is mediated via DAergic and serotoninergic systems as well as antioxidant activity in OVX mice. The potential significance of hesperidin suggests that it may have a substantial impact on neurophysiology and neuroprotection which further researches are needed to clarify the precise molecular mechanisms involved in the antidepressant effects of the hesperidin.

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## Disclosure of potential conflicts of interest

Authors have no potential conflicts of interest

## Informed Consent

There is no informed consent in this study.

## Research involving human participants and/or animals

This manuscript does not contain any studies with human subjects performed by any of the authors. All experiments were executed according to the Guide for the Care and Use of Laboratory Animals and were approved by the institutional animal ethics committee.

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