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Anti-depressant effect of Artemisia dracunculus extract mediates via GABAergic and serotoninergic systems in ovariectomized mice

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Abstract

Background and Objective: Depression known as most prevalent mental disorders and there is growing interest to use medical plants with lower side effects. So, the aim of current study was to determine anti-depressant effect of Artemisia dracunculus extract (ADE) in ovariectomized (OVX) mice and its possible interaction with opioidergic, GABAergic and serotoninergic systems.

Materials and Methods: In experiment 1, mice kept as control and sham groups, OVX, OVX+ ADE (12.5 mg/kg), OVX+ ADE (25 mg/kg) and OVX+ADE (50 mg/kg). In experiment 2, mice kept as control and sham, OVX, OVX+ ADE (50 mg/kg), OVX+naloxone (2 mg/kg) and OVX+injection of ADE and naloxone. Experiments 3-5 were similar to experiment 2, except injections of the flumazenil (5 mg/kg), fluoxetine (5 mg/kg) and cyproheptadine (4 mg/kg). 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.

Results: According to the findings, OVX increased immobility time compared to control group ($P<0.05$). ADE (50 mg/kg) decreased depression induced immobility time compared to OVX group ($P<0.05$). Injection of ADE+flumazenil decreased depression induced immobility time in TST and FST and increased number of crossing in OFT ($P<0.05$). Injection of ADE+fluoxetine decreased depression induced immobility time ($P<0.05$). ADE (25 and 50 mg/kg) reduced the MDA while elevated SOD and GPx levels in OVX mice ($P<0.05$).

Conclusion: It seems, antidepressant activity of Artemisia dracunculus mediates via GABAergic and serotoninergic systems in OVX mice.

Keywords: Anti-depressant, Ovariectomy, Mice, Artemisia dracunculus, GABAergic and serotoninergic system

1. Introduction

Depression known as most prevalent mental disorders with symptoms such as psychosocial and physical disorders (1). The major depressive disorder (MDD) alters brain physiological function, emotional and cognitive processes ant its rate of risk is higher in women because of hormonal imbalance in the menopausal stage (2). Ovarian hormones have deniable role in emotional status, mood regulation and cognition. Changes in estradiol levels during the menstrual cycle, parturition and menopause enhance depression risk and mood disruption (3). Based on animal method studies, OVX in mice leads to depressive-like behavior and estradiol therapy showed antidepressant-like effect (4). Antidepressant drugs such as selective serotonin reuptake inhibitors (SSRIs) and tricyclic

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anti-depressants affecting routinely used for treatment of depression (5). However, sedation, blurred vision, constipation, seizures and sexual dysfunction are known as main side effects (5). Thus, there is a growing interest for new medical plant with antidepressant activity and lower side effects.

Many natural compounds and products are potential antioxidants that protect against oxidative damage in chronic diseases. The biological effect of medicinal plant extracts in preventing oxidative damage has been previously reported and the mechanism of action of medicinal extracts has been explained. Artemisia dracunculus L. (Asteraceae) belongs to Asteraceae family which distribution in Eurasia and North America (6). The herb is traditionally used for laxative, eupeptic, carminative, stomachic, antispasmodic, vermifuge, emmenagogue and to treat gastritis (7). Various phytochemical and components of the essential oil are menthol, anethole, anisole, anisic acid, d-sabinene, estragole, limonene, myrcene, ocimene, a-phellandrene, anisaldehyde, α-and β-pinene (8). It is revealed essential oil of chamomile (Anthemis nobilis), clary (Salvia sclarea), rosemary (Rosmarinus officinalis), and lavender (Lavandula angustifolia) had antidepressant effects (9). It is reported OVX-induced depression mediates via nitrergic, serotonergic and dopaminergic (DAergic) systems (10, 11) and herbal plants impress anti-depressive effect via these systems (12).

However, there is limited information about antidepressants of the Artemisia dracunculus in OVX mice. Thus, the aim of current study was to determine anti-depressant effect of ADE in OVX mice and its possible interaction with opioidergic, GABAergic 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 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) (13) (IR.IAU.SRB.REC.1399.1137).

2.2. Drugs

Naloxone (opioid receptor antagonist), flumazenil (GABA receptor antagonist), fluoxetine (selective serotonin reuptake inhibitor) and cyproheptadine (serotonergic receptor antagonist) were purchased from Sigma Aldrich, (St, USA).

2.3. Extraction

Artemisia dracunculus L. were heated with 80% ethanol (v/v) to 80 ° C for 2 h. The extraction continued for an additional 10 h at 20°C. The extract was then filtered through cheese cloth and evaporated with a rotary evaporator. The aqueous extract was freeze-dried for 48 h, and the dried extract was homogenized with a mortar and pestle (14).

2.4. Experimental OVX

Following anesthesia by intraperitoneal (i.p.) injection of ketamine hydrochloride (50 mg/kg) and xylazine hydrochloride (5 mg/kg) (Alfasan, Woerden, and 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 (15, 16). All behavioral tests were done 10 days after recovery (10).

2.5. Experimental procedure

Then OVX mice randomly allocated into 5 experimental groups (n=48 in each experiment and 8 mice in each group). In experiment 1, (A) control group without surgery and injected saline (10 ml/kg) with at 1 h prior the test, (B) sham group had no OVX and injected with saline (10 ml/kg) with at 1 h prior the test, (C) OVX then i.p injected with saline (10 ml/kg) with at 1 h prior the test, (D) OVX then i.p injected with ADE (12.5 mg/kg) at 1 h prior the test, (E) OVX mice injected with ADE (25 mg/kg) at 1 h prior the test and (F) OVX then i.p injected with ADE (50 mg/kg) at 1 h prior the test. In experiment 2, (A) control without surgery and injected saline (10 ml/kg) with at 1 h prior the test, (B) sham had no ovariectomy (OVX) and injected with saline (10 ml/kg) with at 1 h prior the test, (C) OVX then i.p injected with saline (10 ml/kg) with at 1 h prior the test, (D) OVX then i.p injected with ADE (50 mg/kg) at 1 h prior the test, (E) OVX mice injected with naloxone (10 mg/kg) at 1 h prior the test and (F) OVX mice injected with ADE (50 mg/kg) at 1 h prior the test, 15 minutes after final injection animals i.p injected with naloxone (10 mg/kg) and 45 minutes later tests were done. Experiments 3- were similar to experiment 3, except flumazenil (5 mg/kg), fluoxetine (5 mg/kg) and cyproheptadine (4 mg/kg) instead of naloxone. Then FST, TST and OFT tests were done. At the end of the study, serum MDA, SOD, GPx and TAS levels were determined.

2.6. Forced Swimming Test

The FST was done using the described protocol in mice (17). Animal was plunged into a glass cylinder containing 25 ± 1 ℃ 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'.

2.7. Tail Suspension Test

It is knowns as common antidepressant-like activity in mice (18). 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.

2.8. Open field test

The OFT was used to determine the possible effects of ADE on the locomotor and exploratory activities. The open-field was done using a 45×45×30 cm3 poly wood cage. The floor of the open field cage was divided by masking tape markers into 3×3 cm2 squares. Mice was placed individually observed for 6 min for number of segments crossed with the four paws (19).

2.9. Antioxidant activity

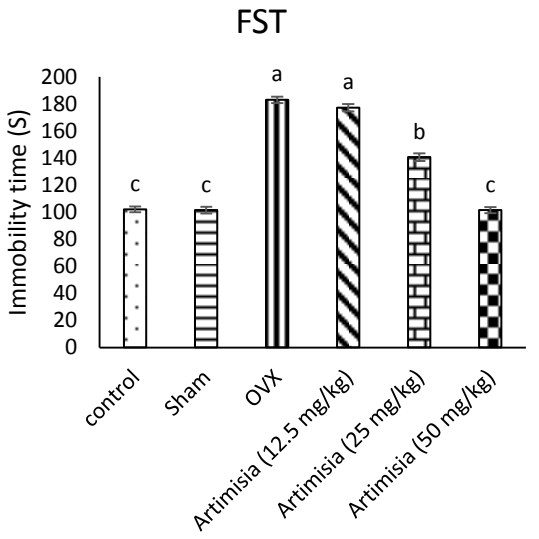
At the end of the tests, blood samples were collected via cardiac puncture and serum MDA, SOD, GPx and total antioxidant capacity (TAC) were determined by ELISA method using Zell Bio GmbH (Germany) commercial assay kits.

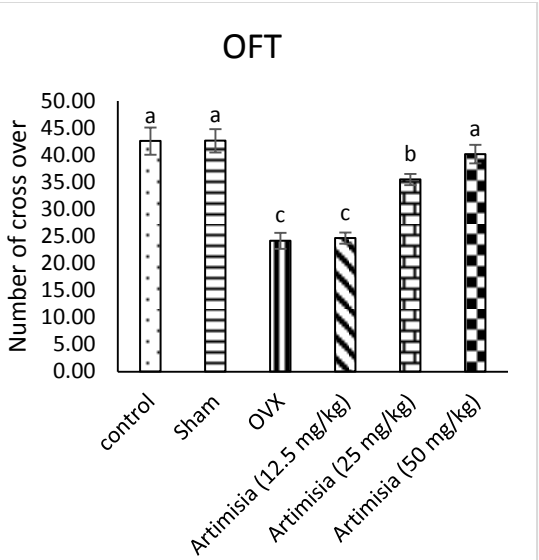
2.10. Statistical analysis

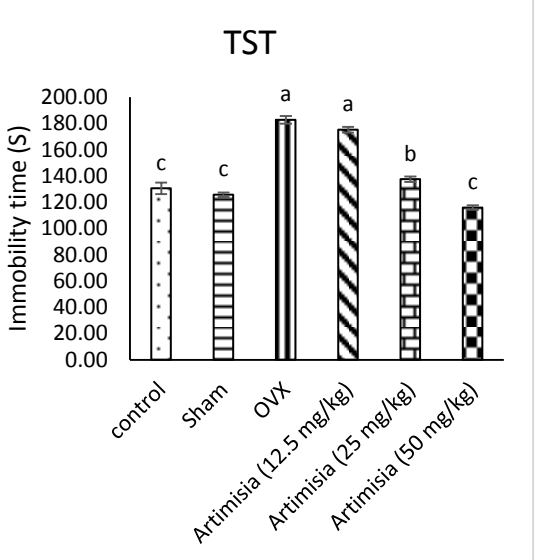
Data was analyzed by one-way analysis of variance (ANOVA). For treatments found to have an effect according to the ANOVA, mean values were compared with Tukey's test. Data is presented as the mean ± SEM and $p < 0.05$ were considered to indicate significant difference.

3. Results

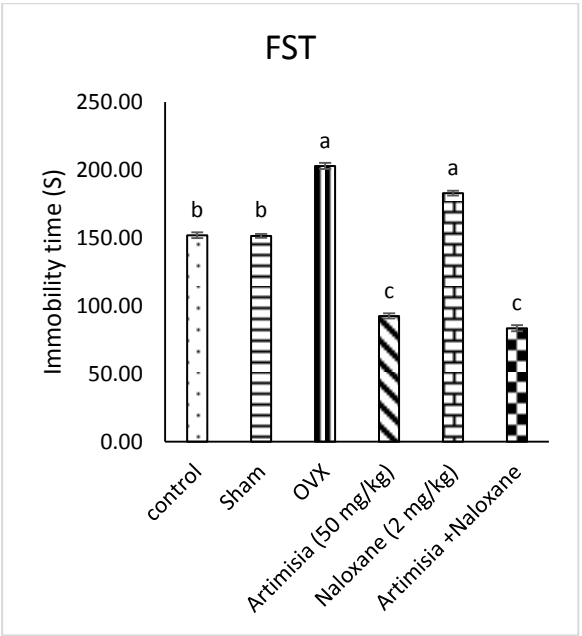
As seen in figure 1, no significant difference observed on immobility time in FST and TST tests in sham compared to control group ($P>0.05$). The OVX significantly increased immobility time in FST and TST tests compared to control group ($P<0.05$). ADE in a dose dependent manner decreased depression induced immobility time in comparison to OVX group ($P<0.05$). ADE (25 and 50 mg/kg) significantly increased the number of crossing in OFT test in comparison to OVX mice ($P<0.05$).

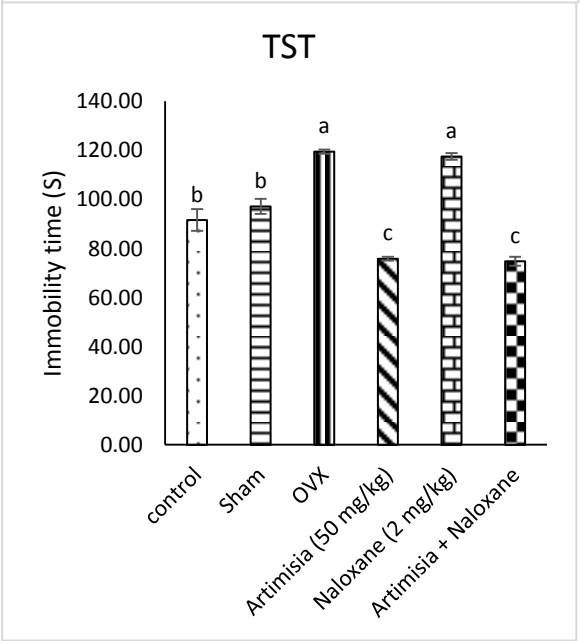






According to the figure 2, no significant difference observed on immobility time in FST and TST tests in control and sham groups (P>0.05). The OVX significantly increased immobility time in FST and TST tests compared to control mice (P<0.05). ADE (50 mg/kg) significantly reduced depression induced immobility time in comparison to OVX group (P<0.05). Naloxone had no effect on immobility time in TST and FST and number of crossing in OFT (P>0.05). Injection of ADE+naloxone had no effect on immobility time in TST and FST and number of crossing in OFT (P>0.05).





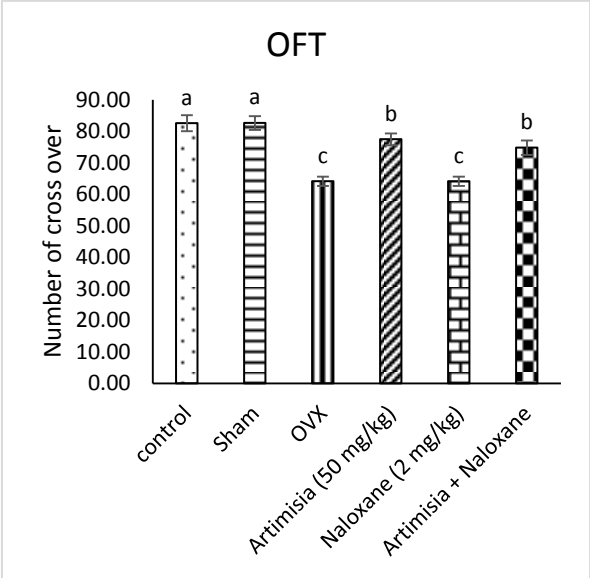
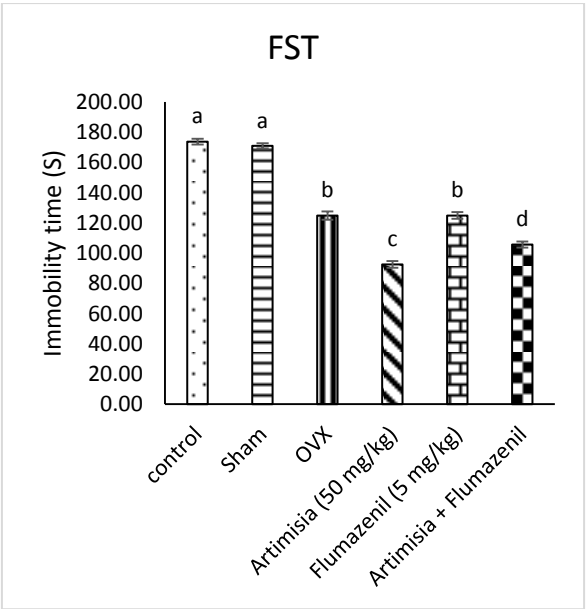


Figure 2. Effects of Artemisia dracunculus (50 mg/kg), Naloxone (2 mg/kg) and their co-injection on FST (Up), TST (Middle) and OFT (Down) tests in ovariectomized mice. Different letters (a-d) indicate significant differences between treatments (P<0.05). TST: tail suspension test, FST: forced swimming test, OFT: open field.

Injection of the ADE (50 mg/kg) + flumazenil (5 mg/kg) significantly decreased the antidepressant activity of the ADE compared to OVX group (P<0.05). The OVX significantly reduced the number of crossing compared to OVX mice (P<0.05). The ADE (50 mg/kg) significantly increased number of crossing comparison to OVX mice (P<0.05). Co-injection of the ADE (50 mg/kg) + flumazenil (5 mg/kg) decreased the positive effect of the ADE on number of crossing (P<0.05). It seems, the antidepressant activity of the ADE mediates via GABAergic system in OVX mice (figure 3).



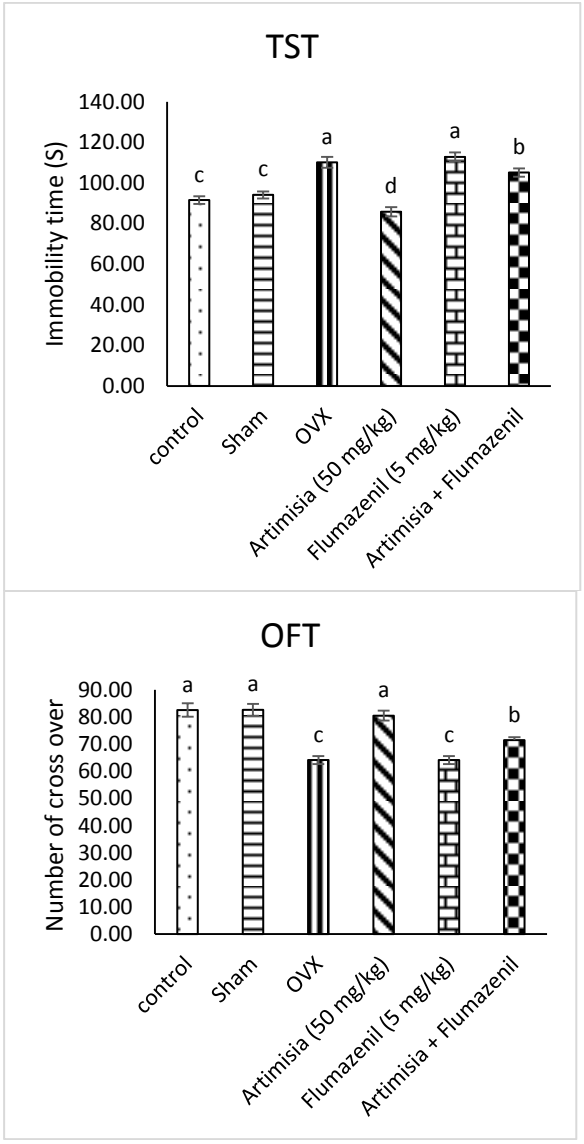


Figure 3. Effects of Artemisia dracunculus (50 mg/kg), Flumazenil (5 mg/kg) and their co-injection on FST (Up), TST (Middle) and OFT (Down) tests in ovariectomized mice. Different letters (a-d) indicate significant differences between treatments (P<0.05). TST: tail suspension test, FST: forced swimming test, OFT: open field.

As seen in figure 4, OVX significantly increased immobility time in FST and TST tests compared to control mice (P<0.05). ADE (50 mg/kg) significantly lessened immobility time (P<0.05). Fluoxetine (5 mg/kg) had no effect on immobility time in TST and FST and number of crossing in OFT (P>0.05). Injection of the ADE (50 mg/kg) + fluoxetine (5 mg/kg) significantly decreased immobility time in FST and TST tests (P<0.05). The OVX significantly reduced the number of crossing compared to OVX mice (P<0.05). The ADE (50 mg/kg) significantly increased number of crossing comparison to OVX mice (P<0.05). Co-injection of the ADE (50 mg/kg) + fluoxetine (5 mg/kg) amplified the positive effect of the ADE on number of crossing in comparison to OVX group (P<0.05). It seems, the antidepressant activity of the ADE mediates via serotoninergic system in OVX mice (figure 4).

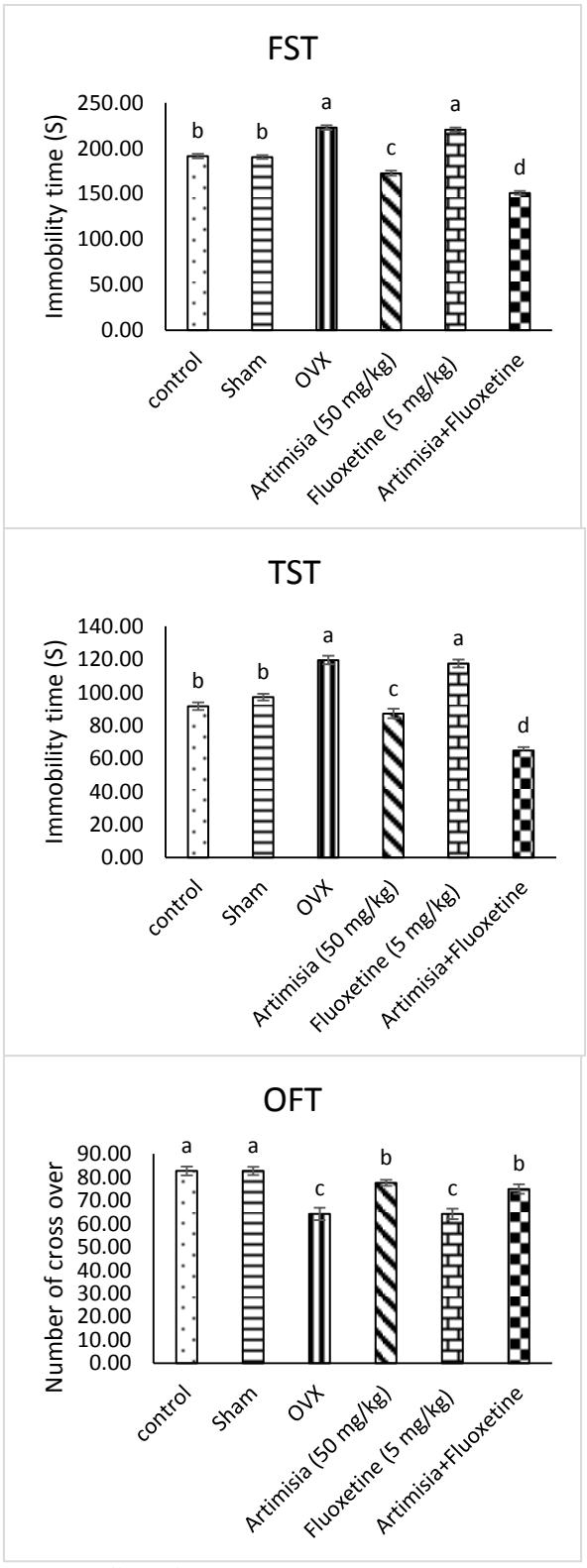
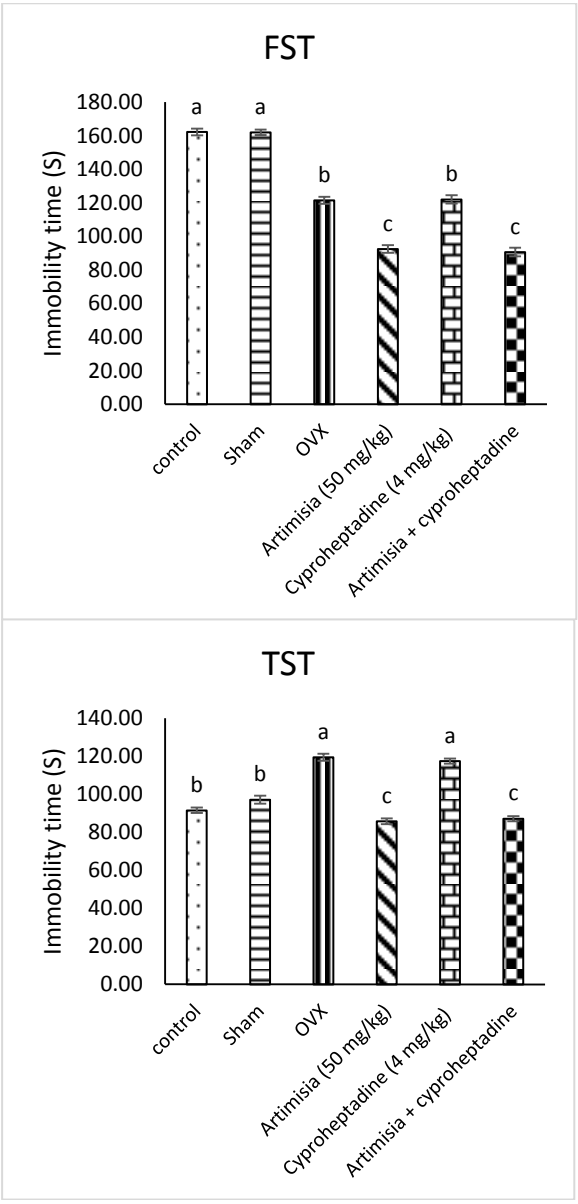


Figure 4. Effects of Artemisia dracunculus (50 mg/kg), Fluoxetine (5 mg/kg) and their co-injection on FST (Up), TST (Middle) and OFT (Down) tests in ovariectomized mice. Different letters (a-d) indicate significant differences between treatments (P<0.05). TST: tail suspension test, FST: forced swimming test, OFT: open field.

According to the figure 5, no significant difference observed on immobility time in FST and TST tests in 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). ADE (50 mg/kg) significantly reduced depression induced immobility time in comparison to OVX group (P<0.05). Cyproheptadine (4 mg/kg) had no effect on immobility time in TST and FST and number of crossing in OFT (P>0.05). Injection of ADE + cyproheptadine had no effect on immobility time in TST and FST and number of crossing in OFT (P>0.05).



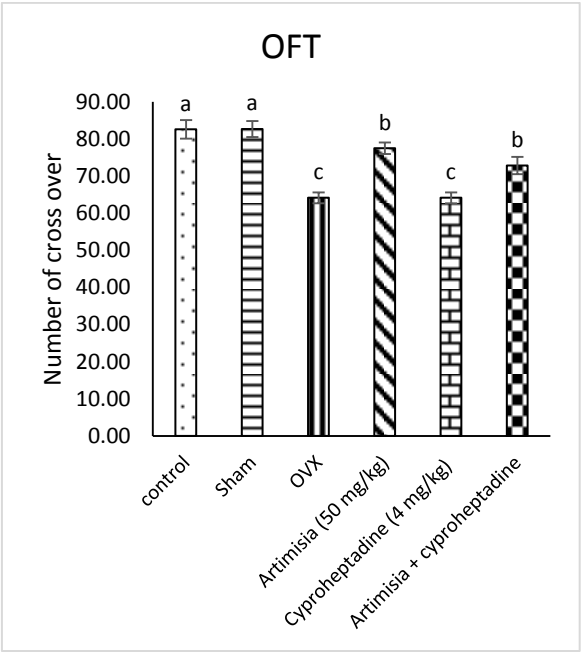


Figure 5. Effects of Artemisia dracunculus (50 mg/kg), Cyproheptadine (4 mg/kg) and their co-injection on FST (Up), TST (Middle) and OFT (Down) tests in ovariectomized mice. Different letters (a-d) indicate significant differences between treatments (P<0.05). TST: tail suspension test, FST: forced swimming test, OFT: open field.

As seen in table 1, OVX significantly increased MDA levels compared to the control group (P<0.05). ADE (25 and 50 mg/kg) significantly decreased the MDA levels compared to the OVX mice (P<0.05). The SOD and GPx levels significantly diminished following OVX (P<0.05). ADE (25 and 50 mg/kg) significantly elevated the SOD and GPx levels in comparison to the OVX mice (P<0.05). However, no significant fluctuation on TAS was observed (P>0.05).

Table 1. Effect of different levels *Artemisia dracunculus* 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	56 ± 0.24 [c]	849 ± 2.13 [a]	89 ± 0.26 [a]	230 ± 12.02
Sham	60 ± 0.32 [c]	854 ± 2.11 [a]	85 ± 0.16 [a]	221 ± 10.01
OVX	91 ± 0.11 [a]	561 ± 1.11 [d]	64 ± 0.11 [d]	185 ± 20.04
Artemisia (12.5 mg/kg)	88 ± 0.25 [a]	577.65 ± 1.16 [d]	66 ± 0.23 [d]	195 ± 14.02
Artemisia (25 mg/kg)	76 ± 0.13 [b]	631.66 ± 1.21 [c]	75 ± 0.16 [c]	200 ± 15.03
Artemisia (50 mg/kg)	66 ± 0.24 [c]	751.53 ± 1.18 [b]	81 ± 0.11 [b]	202 ± 16.01

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

The aim of current study was to determine anti-depressant effect of ADE in OVX mice and its possible interaction with opioidergic, GABAergic and serotoninergic systems. As observed, OVX increased immobility time and ADE (50 mg/kg) decreased depression induced immobility time. Injection of ADE + flumazenil or ADE + fluoxetine decreased depression induced immobility time in TST and FST and increased number of crossing in OFT. ADE (25 and 50 mg/kg) reduced the MDA while elevated SOD and GPx levels in OVX mice. Depression and anxiety are widespread psychological conditions with broad health implications. The current antidepressant therapies are primarily designed to target the serotonergic and/or noradrenergic system in the brain (20). Sex-hormone-dependent depression is a serious problem that Its frequency is higher in women than the men. Estrogen has effects on modulating mood and emotions (21). There is a correlation between mood and estrogen levels since lower estrogen levels increase mood disorders in women (22). However, almost half of patients do not respond to antidepressants, and these interventions are often associated with a wide range of adverse side effects (23). Depression can be caused by an imbalance of neurotransmitters such as serotonin, adrenaline, dopamine and glutamate with their receptors (24). Depressive-like behavior (due to OVX) can be relieved by estradiol (25).

As seen OVX significantly increased immobility time in FST and TST. ADE in a dose dependent manner decreased depression induced immobility time in comparison to OVX group. ADE (25 and 50 mg/kg) significantly increased the number of crossing in OFT. It is reported Artemisia dracunculus aqueous extract improves blood sugar, serum insulin, insulin resistance index and liver enzymes in rat model (26). Also, artemisinin at the level of the 10 mg/kg have anti-nociceptive effects on the inflammatory pain (27). Also, essential oil of Artemisia dracunculus (10, 30, 100 and 300 mg/kg) significantly inhibited (89, 95, 97 and 97%) the nociception produced by acetic acid (6). Also, Artemisia dracunculus promotes psychological resilience in depressed mice (20).

According to the findings, naloxone had no effect on immobility time in TST and FST and number of crossing in OFT. Injection of ADE + naloxone had no effect on immobility time in TST and FST and number of crossing in OFT. Based on the results, flumazenil (5 mg/kg) had no effect on immobility time in TST and FST and number of crossing in OFT. Injection of the ADE (50 mg/kg) + flumazenil (5 mg/kg) significantly decreased the antidepressant activity of the ADE on immobility time. It seems, the antidepressant activity of the ADE mediates via GABAergic system in OVX mice. As seen fluoxetine (5 mg/kg) had no effect on immobility time in TST and FST and number of crossing in OFT. Injection of the ADE (50 mg/kg) + fluoxetine (5 mg/kg) significantly decreased the antidepressant activity of the ADE on immobility time compared to OVX group. ADE (50 mg/kg) significantly increased number of crossing comparison to OVX mice (P<0.05). It seems, the antidepressant activity of the ADE mediates via serotoninergic system in OVX mice. Injection of ADE + cyproheptadine had no effect on immobility time in TST and FST and number of crossing in OFT.

Depression is associated with the hyperactivity of immune inflammatory responses as manifested by elevated expression of pro-inflammatory molecules, such as IL-6 and TNF-α (24). ADE have high content of secondary metabolites, including coumarins, flavonoids, and phenylpropanoid acids which decrease stress-induced depression in mice. Also, ADE reduction of inflammatory cytokines in the blood (28). It is assumed anti-depressive activity of the ADE mediates via opioidergic system. It has been proposed

that involvement of the opioid system in the antidepressants' mechanism of action is critical for treatment of severe depression (29). In addition, β-endorphin-positive cell bodies in the arcuate nucleus project to the paraventricular nucleus suggests that β-endorphin has important physiological role in stress response. In the current study, ADE decreased depression in OVX mice and perhaps observed results might related to its antioxidant activity. Previous studies revealed that increased glutamatergic transmission on ventral striatum medium spiny neurons mediates stress-induced susceptibility following repeated social defeat stress (30). It is reported flavonoid (chlorogenic acid) isolated from the Artemisia capillaris Thunb. Has antidepressant activities in FST, TST and rotarod test in mice models of depression (29). The TST and FST are routinely used for antidepressants. Antidepressants reduce the immobility time in both TST and FST.As seen OVX significantly increased MDA levels and ADE (12.5, 25 and 50 mg/kg) significantly decreased the MDA levels compared to the OVX mice. The SOD and GPx levels significantly diminished following OVX a ADE (12.5, 25 50 mg/kg) significantly elevated the SOD and GPx levels in comparison to the OVX mice. Artemisia has protective effect against reactive oxygen species (ROS) production and oxidative stress. Hesperidin increase antioxidant enzymes CAT, SOD and glutathione S-transferase (GST) level (9). The CAT, SOD, GPx and GST play important role on scavenging 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 (31). The positive role of the antioxidant effects of antidepressants in the treatment of major depressive disorder is well documented. Antunes et al. (32) reported antioxidant activity of may be mediating by O2– and modulation of the CAT and GPx activity. Previous reports revealed antioxidants prevented cell membrane damage in neurodegenerative diseases (33). It seems, antidepressant activity of ADE might have related to its antioxidant activity and mediates via GABAergic and serotoninergic system in OVX mice.

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Conflicts of interest

Authors have no potential conflicts of interest

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