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Original Article

Anti-depressant effect of *Oliveria Decumbens* in gonadectomized rat

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ABSTRACT

This study aimed to determine the antidepressant effect of *Oliveria Decumbens* extract (ODE) in gonadectomized rats. Thirty male rats were allocated into 6 experimental groups. The control group underwent identical surgeries, but the testes were not clamped, ligated, or excised. In the imipramine group, castration was identical, followed by imipramine administration (5 mg/kg) for 2 weeks. In groups 4, 5, and 6, following castration, rats were p.o. injected with ODE (100, 200, and 400 mg/kg) for 2 weeks, respectively. Then, the forced swimming test (FST), the tail suspension test (TST), and the open field test (OFT) were done. Also, serum levels of malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (GPx), and total antioxidant status (TAS) were determined. According to the data, immobility time using FST and TST significantly increased in the gonadectomized rat ($p< 0.05$). Administration of imipramine significantly decreased immobility time using FST and TST ($p< 0.05$). A dose-dependent decrease in immobility time in the FST and TST was observed in gonadectomized rats treated with ODE (200 and 400 mg/kg) compared with the control group ($P<0.05$). Castration significantly decreased the number of crosses in OFT, and administration of imipramine or treatment with ODE (200 and 400 mg/kg) significantly increased it ($p< 0.05$). Serum MDA levels significantly increased, while SOD, GPx, and TAS levels decreased in gonadectomized rats compared with the control group ($p< 0.05$). Administration of the ODE (200 and 400 mg/kg) significantly decreased serum MDA while increasing SOD, GPx, and TAS levels ($p< 0.05$). These results suggest that ODE has an antidepressant effect in gonadectomized rats, mediated by its antioxidant activity.

اثرات ضدافسردگی گیاه لعل کوهستان در موش های گنادکتومی شده

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چکیده

این مطالعه با هدف تعیین اثر ضد افسردگی عصاره لعل کوهستان در موش های صحرایی گنادکتومی شده انجام شد. ۳۰ سر موش صحرایی نر در ۶ گروه آزمایشی قرار گرفتند. در گروه کنترل جراحی هایی صرفا ایجاد جراحت بدون اخته کردن بود. در گروه ایمی پرامین متعاقب اخته کردن ایمی پرامین (۵ میلی گرم بر کیلوگرم) به مدت ۲ هفته تجویز شد. در گروه‌های ۴، ۵ و ۶ به دنبال اخته کردن، موش‌های صحرایی تجویز دهانی عصاره لعل کوهستان (۱۰۰، ۲۰۰ و ۴۰۰ میلی گرم بر کیلوگرم) به ترتیب به مدت ۲ هفته را دریافت کردند. سپس تست شنای اجباری (FST)، تست تعلیق دم (TST) و تست میدان باز (OFT) انجام شد. همچنین سطوح سرمی مالون دی آلدئید (MDA)، سوپراکسید دیسموتاز (SOD)، گلوتاتیون پراکسیداز (GPx) و وضعیت آنتی اکسیدانی کل (TAS) تعیین شد. براساس داده‌ها، زمان بی‌حرکتی در آزمون های FST و TST در موش‌های گنادکتومی شده به طور معنی‌داری افزایش یافت ($p<$ ۰/۰۵). تجویز ایمی پرامین به طور قابل توجهی زمان بی حرکتی در تست های FST و TST کاهش داد ($p<$ ۰/۰۵). موش‌های گنادکتومی شده تحت درمان عصاره لعل کوهستان (۲۰۰ و ۴۰۰ میلی گرم بر کیلوگرم) کاهش وابسته به دوز در زمان بی‌حرکتی FST و TST نسبت به گروه کنترل مشاهده شد ($p<$ ۰/۰۵). اخته کردن به طور معنی داری تعداد دفعات عبور در تست OFT را کاهش داد و تجویز ایمی پرامین یا تیمار با سطوح ۲۰۰ و ۴۰۰ میلی گرم بر کیلوگرم عصاره لعل کوهستان به طور معنی داری آن را افزایش داد ($p<$ ۰/۰۵). در موش‌های گنادکتومی شده سطح سرمی MDA به طور معنی‌داری افزایش یافت، در حالی که سطوحSOD ، GPx و TAS نسبت به گروه کنترل کاهش یافت ($p<$ ۰/۰۵). تجویز عصاره لعل کوهستان (۲۰۰ و ۴۰۰ میلی گرم بر کیلوگرم) به طور قابل توجهی MDA سرم را کاهش داد در حالی که سطوحSOD ، GPx و TAS را افزایش داد ($p<$ ۰/۰۵). این نتایج نشان داد که عصاره لعل کوهستان اثر ضد افسردگی دارد و این اثر به واسطه فعالیت آنتی اکسیدانی آن در موش های گنادکتومی شده انجام می شود.

واژه های کلیدی: ضد افسردگی، لعل کوهستان، گنادکتومی، موش

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INTRODUCTION

Depression and anxiety disorders are the prevalent emotional disorders linked to biochemical, cognitive, behavioral, and psychological alterations in the brain in both genders [1]. Depression is linked to hormonal changes, which affect the hypothalamic-pituitary-adrenal [2]. Depression is linked to increased levels of cortisol and corticotropin-releasing hormone. Imipramine, known as a norepinephrine reuptake inhibitor, is commonly recommended for patients because of its efficacy and affordability [3]. Owing to the side effects of imipramine, there is increasing interest in utilizing new antidepressants that have comparable therapeutic efficacy but reduced side effects [4]. Testosterone therapy in castrated males enhances depressive symptoms, but in cases like prostate cancer, it is not an ideal option for treating depression. Additionally, cellular antioxidant enzymes such as SOD, CAT, and GPx are reduced in the blood of rats following orchiectomy-induced depression [5]. Consequently, there is increasing interest in using herbal treatments with fewer side effects [6]. Testicular androgens are essential for physiological equilibrium, influence health outcomes, and contribute to disease mechanisms. The rate of major depressive disorder is double in women compared to men, indicating that physiological testosterone levels may reduce depression risk [7]. Preclinical data further suggest that androgens might mitigate depression in males due to their antidepressant and neuroprotective effects in mood-regulating brain regions such as the hippocampus and limbic system [8]. Extensive evidence shows that low testosterone, clinical hypogonadism, pharmacological androgen deprivation, and androgen receptor antagonist therapy are significantly associated with depression in men, although some studies report no such correlation. An important research question is whether low testosterone serves as a trait marker for depression risk or a state marker for depressive episodes and severity [9]. *Oliveria Decumbens* is an annual herb in the *Apiaceae* family native to Asia. In Iran, known as Mashkourak or Den, which exists in limited areas of the south and west [10]. This herb has been used in traditional or folk medicine to treat various ailments, including indigestion, diarrhea, stomach discomfort, fever, and infections. Previous studies on the essential oil of *Oliveria decumbens* identified thymol and carvacrol as the main constituents [11]. Furthermore, the essential oil's noteworthy antioxidant and antibacterial properties are due to its phenolic compounds [12]. *Oliveria decumbens* has antibacterial, antifungal, anti-inflammatory, and antioxidant activities, as well as cytotoxicity against brine shrimp [10]. Furthermore, it functions as a stimulant for the central nervous system [13]. Thus, this study aimed to determine the antidepressant effect of *Oliveria decumbens* extract (ODE) in gonadectomized rats.

MATERIALS AND METHODS

Animals and the surgery procedure

A total of thirty adult male Wistar rats (weighing 200–250 g) were allocated into 6 experimental groups (n=6/group). Rats were housed in standard plastic cages under laboratory conditions (22 ± 2°C, 12 h light/dark cycle). Food and tap water were freely accessible at all times. A week after the animals adjusted to the new laboratory conditions, the surgical procedure was performed. The incision area was shaved and cleansed with ethanol and Betadine. Ophthalmic salve was applied to the eyes to prevent drying. A 1 cm cut was created with a scalpel in the lower abdomen along the

midline to enter the abdominal cavity. For castration, locking forceps were used to clamp the blood supply to each testis, followed by ligation of the testes with sterile sutures and removal with a scalpel. The layers of muscle and skin were subsequently stitched together, and wound clips were applied to the incision for 8 days to facilitate healing. A further dose of 10 mg/kg meloxicam was administered 24 hours post-surgery [14]. Rats were given time to recuperate and were observed for a week after surgery for indications of discomfort or distress [15].

Extract procedure

*Oliveria decumbens was collect*ed from northern Iran during spring 2024. The samples were then shade-dried and ground at room temperature. For extraction by cold maceration, 20 g of ground leaves were each immersed in methanol and shaken for 24 hours, then filtered through Whatman no. 2 filter paper [16]. The solvent was removed from the filtrate using a rotary vacuum apparatus. The concentrated extract was kept in an oven at 40 °C for 48 hours to ensure purity and complete solvent removal. Finally, extracts were dried, weighed, and stored at 4 °C in a refrigerator for later testing [17].

Experimental groups and treatments

Following recovery, the rats were divided into five experimental groups (n=6 per group). The control group underwent identical surgeries, but the testes were not clamped, ligated, or excised. In the imipramine group, castration was identical, followed by imipramine administration (5 mg/kg) for 2 weeks. In groups 4, 5, and 6, following castration, rats were p.o. injected with ODE (100, 200, and 400 mg/kg, respectively) for 2 weeks.

Behavioral tests

FST

The approach utilized was the one outlined by Boissy [18]. Swimming trials were performed by placing the rat into separate glass cylinders (height = 50 cm, diameter = 30 cm) filled with 30 cm of water maintained at 23 ± 2°C. The experimental phase lasted 6 minutes, with the first 2 minutes designated for acclimatization, and immobility duration was recorded during the last 4 minutes [19].

TST

Animals were hung from a horizontal beam with adhesive tape secured around the end of their tails. The study procedure lasted 6 minutes, with the first 2 minutes for adaptation, and the time spent immobile was recorded during the final 4 minutes [20].

OFT

The locomotor activity was evaluated using an OFT. The experiment was conducted in a cage measuring 45×45×30 cm^3, with the floor sectioned into 3×3 cm^2. Each rat was placed in the middle of the open field. After 2 minutes, the number of squares all four feet crossed was recorded for 4 minutes. Between tests, the area was sanitized with ethanol to eliminate odor bias [21].

Antioxidant assay

After behavioral testing, blood samples were collected from each animal, and serum MDA, SOD, GPx, and CAT were determined by ELISA using Zell Bio GmbH (Germany) assay kits.

Statistical Analysis

Statistics were evaluated with one-way ANOVA, and results were displayed as mean ± SE (standard error) in SPSS version 22.0. Between-group analyses were conducted using the Tukey post hoc test ($p< 0.05$) for treatments that demonstrated significant differences in ANOVA.

RESULTS

According to the results, immobility time in the FST was significantly longer in the gonadectomized rats than in the control group ($p< 0.05$). Administration of imipramine significantly decreased immobility time compared with the control group ($p< 0.05$). Administration of the ODE (100 mg/kg) had no effect on immobility time compared to the control group ($p> 0.05$). A dose-dependent decrease in immobility time was seen in gonadectomized rats treated with ODE (200 and 400 mg/kg) compared to the control group ($p< 0.05$) (Figure 1). As shown in Figure 2, immobility time during TST was significantly longer in gonadectomized rats than in the control group ($p< 0.05$). Administration of imipramine significantly decreased immobility time compared with the control group ($p< 0.05$). No significant difference in immobility time was observed in gonadectomized rats receiving ODE (100 mg/kg) compared with the control group ($p< 0.05$). Administration of the ODE (200 and 400 mg/kg) significantly decreased immobility time compared with the control group ($p< 0.05$). Based on Figure 3, the number of cross-sections in the OFT was significantly lower in the gonadectomized rat than in the control group ($p< 0.05$). Administration of imipramine significantly increased the number of crossings compared with the control group ($p< 0.05$). Administration of the ODE (100 mg/kg) had no effect on the number of crosses compared to the control group ($p> 0.05$). ODE (200 and 400 mg/kg) significantly increased the number of crossings in the OFT compared with the control group ($p< 0.05$). As shown in Figure 4, serum MDA levels were significantly elevated in gonadectomized rats compared with the control group ($p< 0.05$). Administration of imipramine or ODE (100 mg/kg) did not increase serum MDA levels compared with the control group ($p> 0.05$). Administration of the ODE (200 and 400 mg/kg) significantly decreased serum MDA levels compared with the control group ($p< 0.05$). Based on findings, the serum level of SOD significantly decreased in the gonadectomized rats compared to the control group ($p< 0.05$). No significant change was seen in the serum level of the SOD in gonadectomized rats receiving imipramine or ODE (100 mg/kg) compared to the control group ($p< 0.05$). Administration of the ODE (200 and 400 mg/kg) significantly improved the serum level of the SOD in comparison to the control group ($p< 0.05$) (Figure 5). As shown in Figure 6, the serum GPx level was significantly lower in gonadectomized rats than in the control group ($p< 0.05$). No significant change in imipramine or GPx serum levels was observed in gonadectomized rats receiving ODE (100 mg/kg) compared with the control group ($p< 0.05$). Administration of the ODE (200 and 400 mg/kg) significantly increased serum GPx levels compared with the control group ($p< 0.05$). As shown in Figure 7, the serum TAS level was significantly lower in gonadectomized rats than in the control group ($p< 0.05$). Administration of imipramine or ODE (100 mg/kg) had no effect on TAS serum levels compared with the control group ($p> 0.05$). ODE (200 and 400 mg/kg) significantly increased serum TAS levels compared with the control group ($p< 0.05$).

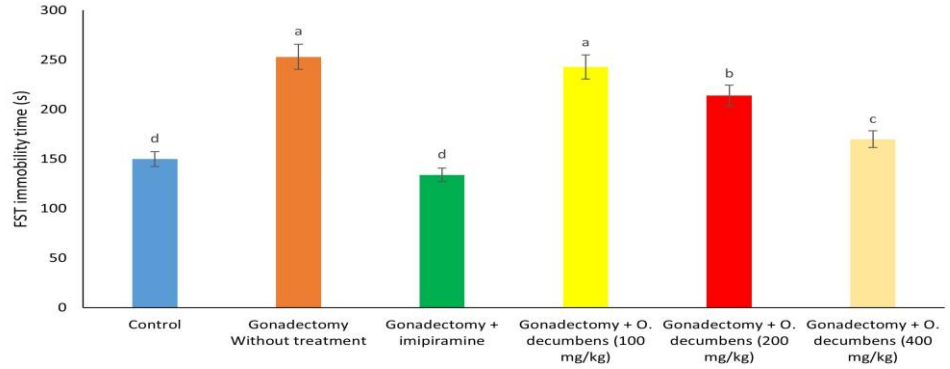

Figure 1: Effect of *Oliveria Decumbens* on immobility time using FST in gonadectomized rat. Data are expressed as mean ± SE. Different superscripts (a-d) indicate significant differences between groups ($p< 0.05$).

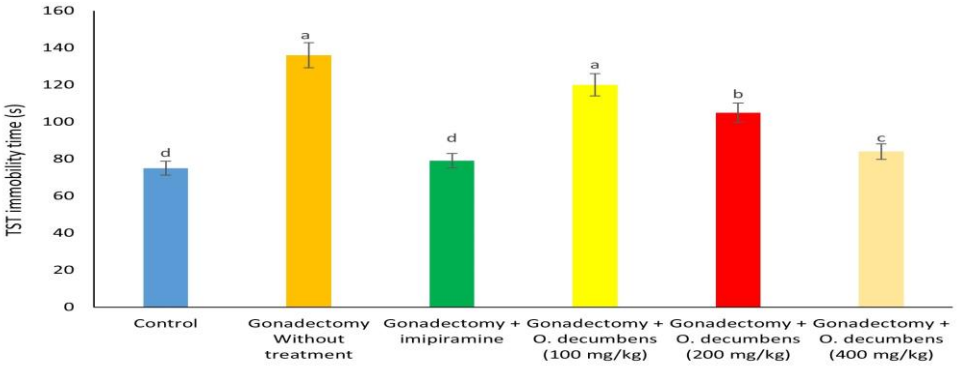

Figure 2: Effect of *Oliveria Decumbens* on immobility time using TST in gonadectomized rat. Data are expressed as mean ± SE. Different superscripts (a-d) indicate significant differences between groups ($p< 0.05$).

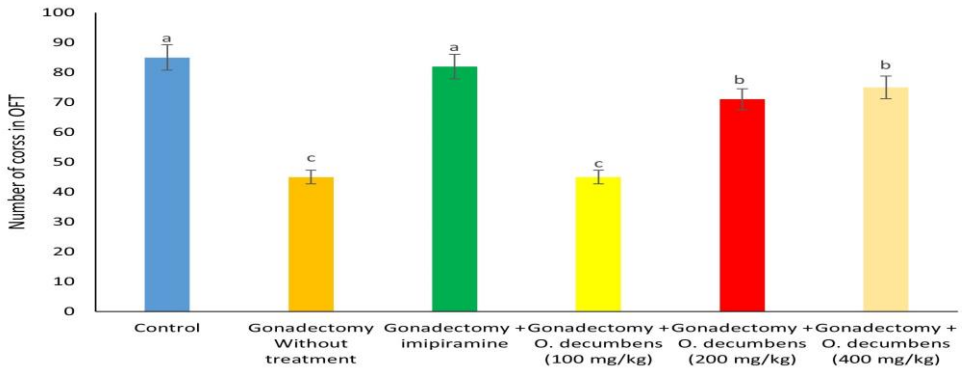

Figure 3: Effect of *Oliveria Decumbens* on number of cross using OFT in gonadectomized rat. Data are expressed as mean ± SE. Different superscripts (a-c) indicate significant differences between groups ($p< 0.05$).

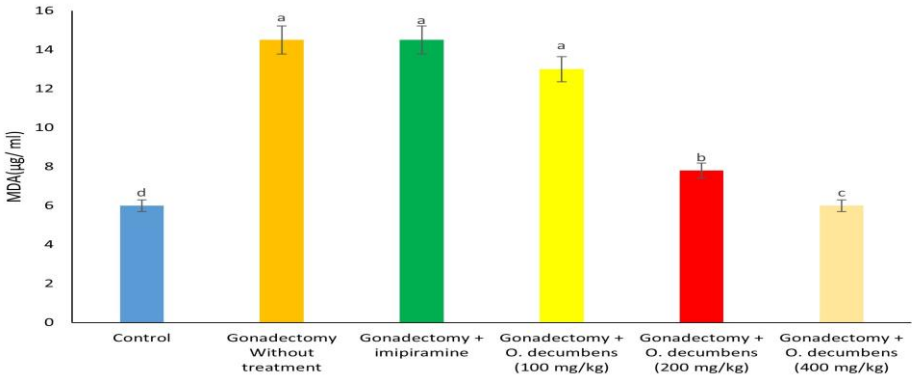

Figure 4: Effect of *Oliveria Decumbens* on serum MDA levels in gonadectomized rat. Data are expressed as mean ± SE. Different superscripts (a-d) indicate significant differences between groups ($p< 0.05$).

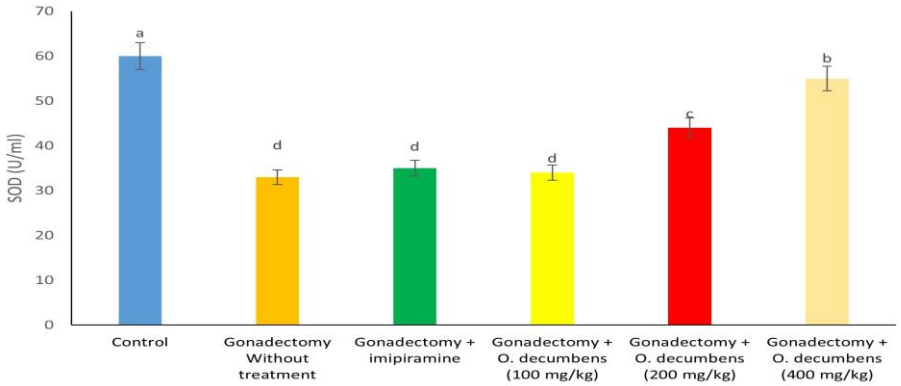

Figure 5: Effect of *Oliveria Decumbens* on serum SOD levels in gonadectomized rat. Data are expressed as mean ± SE. Different superscripts (a-d) indicate significant differences between groups ($p< 0.05$).

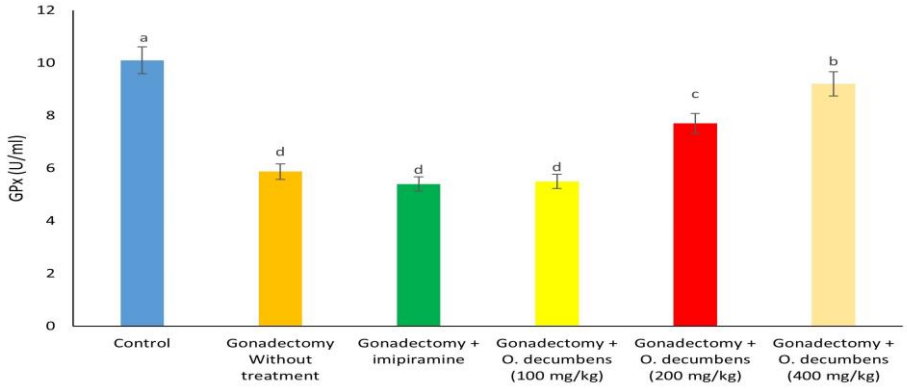

Figure 6: Effect of *Oliveria Decumbens* on serum GPx levels in gonadectomized rat. Data are expressed as mean ± SE. Different superscripts (a-d) indicate significant differences between groups ($p< 0.05$).

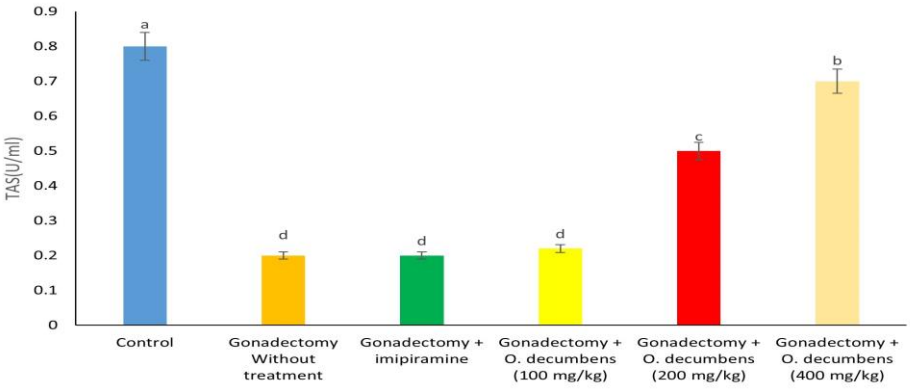

Figure 7: Effect of *Oliveria Decumbens* on serum TAS levels in gonadectomized rat. Data are expressed as mean ± SE. Different superscripts (a-d) indicate significant differences between groups ($p< 0.05$).

DISCUSSION

Based on our main findings, immobility time using FST and TST increased in the gonadectomized rats. Administration of imipramine decreased immobility time using FST and TST. Also, castration decreased the number of crosses in OFT. Depressive disorders are the most common mental health issues associated with biochemical, cognitive, behavioral, and psychological changes [22]. These disorders present in distinct ways for each gender, but the rate of depression disorders in men is approximately 50% lower than in women. Currently, research involving both humans and animals has not yet clarified the neural mechanisms and origins of depression

[23]. A strong connection between a low mood and hypogonadism has been documented. Men suffering from severe and treatment-resistant depression, as well as older men who are depressed, exhibit low testosterone levels [24], and our finding was in agreement with this report. By elevating testosterone levels via endogenous factors or exogenous administration, signs of depression have been found to lessen in individuals. Research has shown a strong connection between dysphoric mood and hypogonadism [25]. Limited studies have assessed the relationship between testosterone and depression. In addition, research has shown that lower levels of testosterone and dehydroepiandrosterone are associated with depression [26]. Imipramine, a norepinephrine reuptake inhibitor, is frequently recommended to patients for its effectiveness and cost-effectiveness [27], and our finding was in agreement with this report. As seen, a dose-dependent decrease in immobility time, FST, and TST was seen in gonadectomized rats treated with ODE (200 and 400 mg/kg). Administration of the imipramine or treatment with ODE (200 and 400 mg/kg), number of crosses in OFT. Serum MDA levels increased, while SOD, GPx, and TAS levels decreased in gonadectomized rats. Administration of the ODE (200 and 400 mg/kg) significantly decreased serum MDA while increasing SOD, GPx, and TAS levels. It is well established that MDA levels and the activities of antioxidant enzymes (SOD, CAT, and GPx) serve as significant biomarkers in anxiety and mood disorders [28]. High serum levels of MDA are associated with anxiety [29]. An excess of ROS or a lack of antioxidants causes oxidative stress in aerobic organisms [5]. Oxidative stress could play a role in the onset of numerous diseases, including cancer, atherosclerosis, myocardial infarction, neurological disorders like Parkinson's and Alzheimer’s, as well as depression [30]. Essential oils are plant extracts commonly used in cosmetics. Essential oils are complex mixtures of hundreds of compounds with varying chemical compositions and amounts. ODE demonstrates hepatoprotective properties against liver damage in rats by reducing oxidative stress [31]. Increased GPx and SOD levels in liver tissue can decrease oxidative stress-mediated injury in cholestatic animal models [32]. Also, ODE therapy in BDL-induced cholestatic rats enhances the activities of SOD and GPx in liver tissue [31], and our findings were consistent with these reports. Because flavonoids inhibit the free radical formation and chelate metal ions by their highly active hydroxyl group, ODE, through regulating antioxidant defense mechanisms, they inhibit the occurrence of depression and oxidative stress in gonadectomized rats. A significant and escalating disease burden highlights the necessity of comprehending the fundamental pathogenic causes and mechanisms of depression. Although there have been scientific advancements in recent years, the biological basis of depression and its reaction to antidepressant therapies remains only partially comprehended [33].

CONCLUSION

These results suggest that ODE has an antidepressant effect, which is mediated by its antioxidant activity, as evidenced by decreased serum MDA levels and increased SOD, GPx, and TAS levels in gonadectomized rats.

ETHICS

Approved.

CONFLICT OF INTEREST

None.

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