Original Article

Effects of Betaine on Ameliorating Depression in Gonadectomized Male Rats

Jina Ghasemi Jalal[1], **Shahin Hassanpour**[2*]

1. Faculty of Veterinary Medicine, Science and Research Branch, Islamic Azad University, Tehran, Iran.
2. Division of Physiology, Department of Basic Sciences, Faculty of Veterinary Medicine, Science and Research Branch, Islamic Azad University, Tehran, Iran.

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ABSTRACT

Background: Depression is a major mental disorder categorized by mood impairment, and betaine has antinociceptive activity in mice.

Objectives This study aimed to determine the antidepressive activity of betaine in gonadectomized male rats.

Methods: Twenty adult male rats were allocated to four experimental groups. Group 1 was kept in the control group, and castration was performed in the other groups. Group 2 was the sham group, which was castrated without treatment. In the imipramine group, the rats were castrated and administered imipramine (15 mg/kg) for two weeks. In group 4, following castration, the rats were intraperitoneally injected with betaine (30 mg/kg) for two weeks, respectively. Antidepressive tests were performed using the forced swimming test (FST), tail suspension test (TST), and open field test (OFT). At the end of the study, blood samples were collected from each cardiac mouse, and serum malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) levels were determined.

Results: Castration significantly increased the immobility time in the FST and TST and the activity of the rats in the OFT ($P<0.05$). Administration of the betaine with castration significantly decreased mobility time on FST and TST and the rat's movement in OFT compared to the untreated group ($P<0.05$). Castration significantly increased serum MDA levels and decreased SOD, GPx, and CAT levels compared to the control group ($P<0.05$). Betaine significantly decreased serum MDA levels and enhanced SOD, GPx, and CAT levels compared to the control group ($P<0.05$).

Conclusion: These results suggest that betaine, a natural antioxidant, is beneficial in decreasing castration-induced depressive behaviors.

Keywords: Betaine, Castration, Depression, Rat

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*** Corresponding Author:**
Shahin Hassanpour, Assistant Professor.
Address: *Division of Physiology, Department of Basic Sciences, Faculty of Veterinary Medicine, Science and Research Branch, Islamic Azad University, Tehran, Iran.*
Phone: *+98 (21) 44868560*
E-mail: *s.hassanpour@srbiau.ac.ir; hassanpour.shahin@gmail.com*

Introduction

Betaine (N, N, N-trimethylglycine) serves as an indispensable provider of methyl groups in the one-carbon metabolic pathway, wherein betaine-homocysteine methyltransferase exclusively employs betaine as a substrate to generate methionine and N, N-dimethylglycine (Arumugam et al., 2021). Betaine plays a pivotal role in osmoregulation and metabolic processes. Betaine supplementation exhibits antioxidative and anti-inflammatory effects, effectively mitigating oxidative stress and nuclear factor-κB, cyclooxygenase-2, and inducible nitric oxide production (Tiwari & Hemalatha, 2022). Numerous studies have evaluated the efficacy of betaine in brain-related disorders. Betaine is promising as a novel psychotherapeutic agent for schizophrenia (Ohnishi et al., 2019). Furthermore, the administration of betaine (30 mg/kg) has alleviated depression induced by zinc oxide toxicity (Jeyhoonabadi et al., 2022). Additionally, betaine exhibits anti-nociceptive activity in mice, and its administration has reduced immobility time in the forced swim test (FST) and elevate serotonin levels in the hippocampus and hypothalamus of rats (Kim et al., 2013).

Depression is a prominent mental disorder characterized by mood impairment, which influences the physiological functioning of the brain and alters emotional and cognitive processes (Zhang et al., 2022). Hormonal fluctuations are closely associated with depression and exert an impact on the hypothalamic-pituitary-adrenal axis. Cortisol and corticotrophin-releasing hormone levels are elevated in individuals with depression. Imipramine, a norepinephrine reuptake inhibitor, is commonly prescribed to patients due to its efficacy and cost-effectiveness (Bangasser & Cuarenta, 2021). Extensive literature has substantiated the significant role of sex hormones in depressive behavior in both sexes. Testosterone depletion is associated with depression, whereas androgen deprivation therapy has ameliorated depressive behavior (Peng et al., 2022). Androgen receptors located in the hippocampus, amygdala, hypothalamus, and cerebral cortex play crucial roles in the pathogenesis of mood and depression. Testosterone can traverse the blood-brain barrier and is present in the regions above. Diminished androgen levels lead to reduced activity in the hippocampus and amygdala, thereby increasing the risk of depression, and testosterone supplementation has been found to improve this condition (Alwhaibi et al., 2022).

Gonadectomy-induced depression in rats results in decreased levels of cellular antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione (Maheshwari et al., 2022). Given the potential side effects associated with imipramine, there is growing interest in the exploration of novel antidepressants with comparable therapeutic efficacy and reduced adverse effects (Silva Almodóvar et al., 2022). Considering the remarkable antioxidative effects of betaine and its role in combating depression, there is currently no report on its antidepressant effect, specifically on gonadectomy-induced behavior in male rats. Therefore, this study aims to investigate the depressant effect of betaine following castration in rats.

Materials and Methods

Animals

Twenty adult male Wistar rats weighing 200-250 g were assigned to four experimental groups, each comprising five rats. The rats were housed in standard plastic cages under laboratory conditions and provided unrestricted access to food and water. Once the animals had acclimated to their environment, a surgical procedure was performed. The surgical site was prepared by shaving the area and cleansing it with ethanol and betadine. A one-centimeter incision was then made with a scalpel in the lower abdomen, running across the midline to access the abdominal cavity. For castration, the blood supply to each testis was clamped using locking forceps, followed by ligation of the testes using sterile sutures and excision with a scalpel. The muscle and skin layers were subsequently sutured, and wound clips were applied over the incision for eight days to facilitate healing. Additionally, meloxicam (10 mg/kg) was administered 24 h after surgery (Huh et al., 2018). The rats were allowed to recover and were closely monitored for signs of discomfort or distress throughout the week following the surgery (Boivin et al., 2017).

Study protocol

After the recovery period, rats were divided into four experimental groups (n=5). The surgeries performed in the control group were identical to those in the other groups, except that the testes were not clamped, ligated, or excised. The Sham group was castrated without further treatment. In the imipramine group, the rats were castrated and subsequently administered imipramine (15 mg/kg) for two weeks. In group 4, the rats were castrated and administered betaine (30 mg/kg) via intraperitoneal injection for two weeks (Ueno et al., 2025).

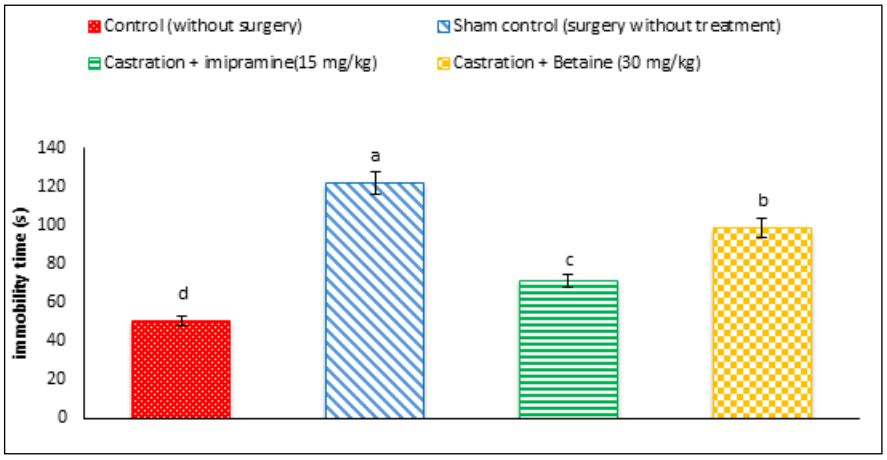

Figure 1. Effects of betaine on immobility time using FST in castrated rats

Note: Different letters (a-d) indicate significant differences between treatments (P<0.05).

Forced swimming test (FST)

The FST involved placing each rat in a glass cylinder filled with water maintained at 23±2 ˚C. The rats were required to swim for six minutes, with the first two minutes as the adaptation period. The duration of immobility was recorded during the final four minutes of the session (Ueno et al., 2025).

Tail suspension test (TST)

In the TST, rats were suspended with adhesive tape wrapped around the tip of their tails on a horizontal beam positioned at a height of 33 cm. After two minutes, the time spent immobile was measured over four minutes (Cryan et al., 2005).

Open field test (OFT)

The rats locomotor behavior was evaluated using an open-field test. The floor of a wooden enclosure measuring 45×45×30 cm^3 was divided into equally sized squares measuring 3×3 cm^2. Each rat was placed in the center of the open-field box, and after two minutes, the number of squares crossed by the animal was recorded during a four-minute observation period (Takeda et al., 1998).

Antioxidant assay

In conclusion, blood samples were collected from each rat's cardiac region, and the levels of serum malondialdehyde (MDA), SOD, GPx, and CAT were determined.

Statistical analysis

The obtained data were subjected to a one-way analysis of variance and are presented as Mean±SE. For treatments exhibiting significant differences, the mean values were compared using the Tukey honestly significant difference (HSD) test (P<0.05).

Results

Figures 1, 2 and 3 show the behavioral changes in castrated rats resulting from betaine administration. As shown in Figure 1, castration significantly increased the immobility time in castrated rats compared to that in the control group during the FST (P<0.05). Pretreatment with imipramine demonstrated a significant decrease in mobility time during the FST test in castrated rats compared to the control group (P<0.05). Administration of betaine in conjunction with castration resulted in a significant decrease in mobility time compared to the untreated group (P<0.05).

Based on the results depicted in Figure 2, castration significantly increased the immobility time in castrated rats compared to the control rats during the TST (P<0.05). Administration of imipramine led to a significant decrease in mobility time compared to the control group (P<0.05). Pretreatment with betaine significantly reduced mobility time compared to the untreated group (P<0.05).

According to the data presented in Figure 3, castration significantly diminished the activity of the rats during the OFT compared to the control group (P<0.05). Pretreatment with imipramine considerably improved the activity of the rats during the OFT test compared to the

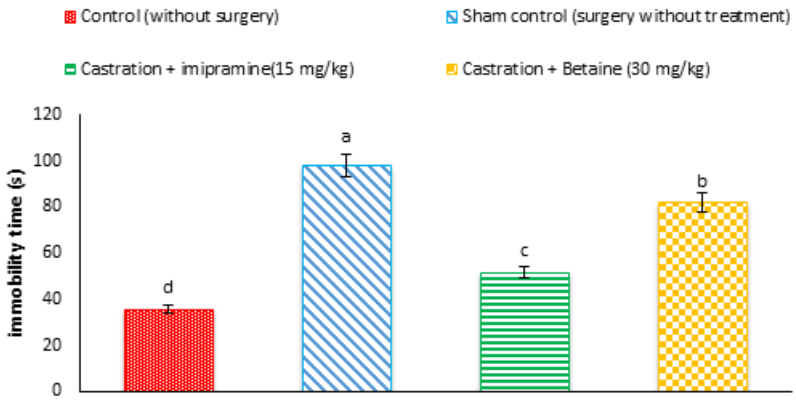

Figure 2. Effects of Betaine on TST in castrated rats

Note: Different letters (a-d) indicate significant differences between treatments (P<0.05).

control group (P<0.05). Administration of betaine significantly increased the movement of the rats during the OFT test in the untreated group (P<0.05).

Figures 4, 5, 6 and 7 show the antioxidant activity of betaine after castration. As depicted in Figure 4, castration significantly increased serum MDA levels compared to the control group (P<0.05). Pretreatment with imipramine significantly decreased serum MDA levels compared to control rats (P<0.05). Betaine administration significantly decreased serum MDA levels compared to the control group (P<0.05).

Based on the data presented in Figure 5, serum SOD levels significantly decreased in the castrated rats than in the control group (P<0.05). Pretreatment with imipramine significantly increased serum SOD levels compared to control rats (P<0.05). Betaine administration significantly increased serum SOD levels compared to the control group (P<0.05).

As shown in Figure 6, serum GPx levels significantly decreased in castrated rats than the control group (P<0.05). Pretreatment with imipramine significantly increased serum GPx levels compared to control rats (P<0.05). The administration of betaine significantly increased serum GPx levels compared to the control group (P<0.05).

In this study, it was observed that serum CAT levels were significantly decreased in rats following castration (P<0.05). Pretreatment with imipramine significantly increased serum CAT levels compared to control rats (P<0.05). The administration of betaine greatly improved CAT levels compared to the control group (P<0.05) (Figure 7).

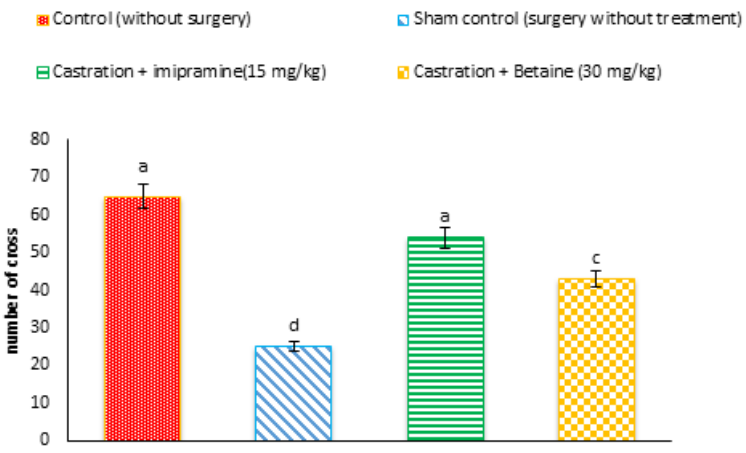

Figure 3. Effects of betaine on OFT in castrated rats

Note: Different letters (a-d) indicate significant differences between treatments (P<0.05).

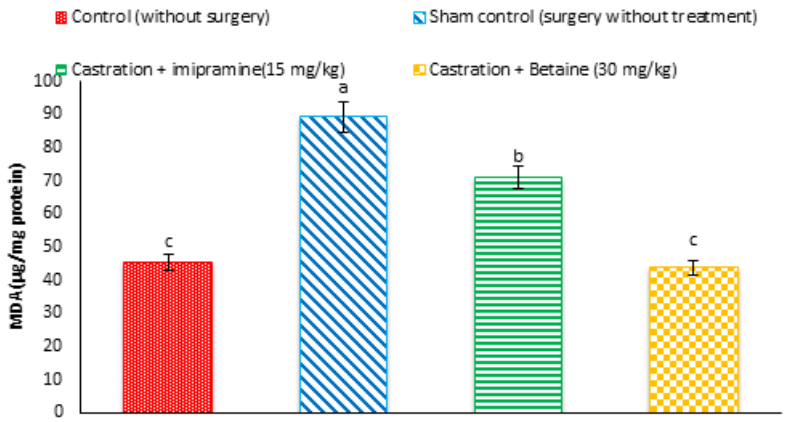

Figure 4. Effects of betaine on serum MDA in castrated rats

Note: Different letters (a-c) indicate significant differences between treatments (P<0.05).

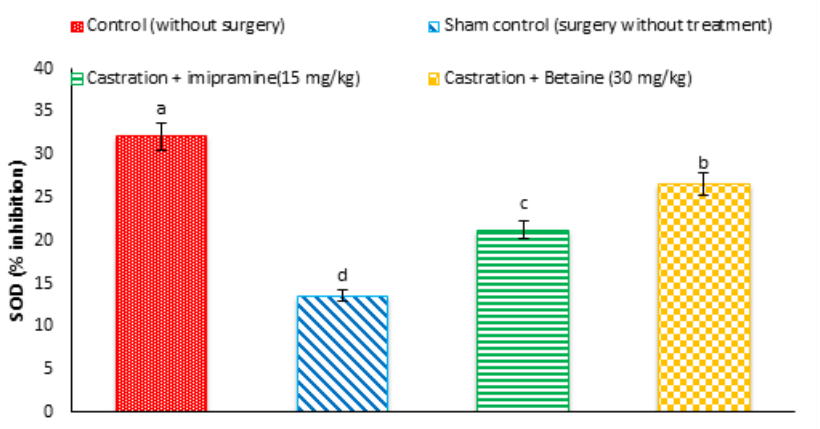

Figure 5. Effects of betaine on serum SOD in castrated rats

Note: Different letters (a-c) indicate significant differences between treatments (P<0.05).

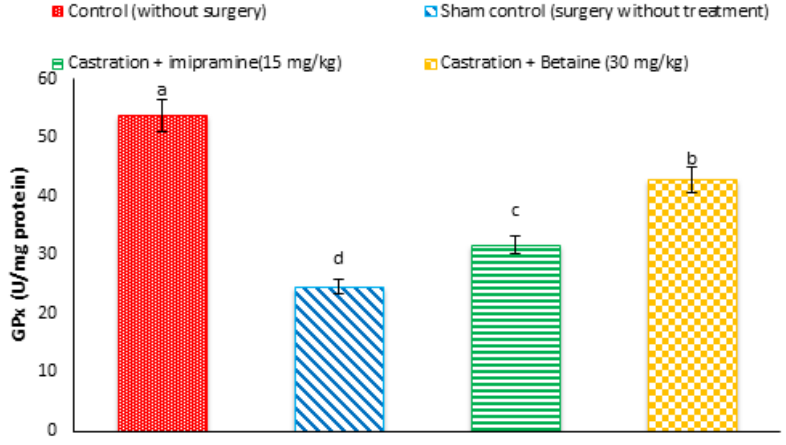

Figure 6. Effects of betaine on serum GPx in castrated rats

Note: Different letters (a-d) indicate significant differences between treatments (P<0.05).

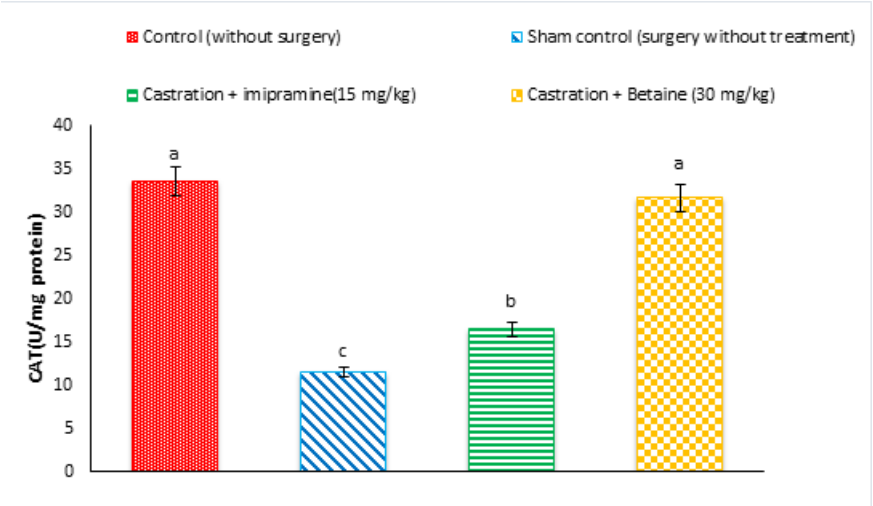

Figure 7. Effects of betaine on serum CAT in castrated rats

Note: Different letters (a-c) indicate significant differences between treatments (P<0.05).

Discussion

Based on the principal results of the present investigation, castration elicited an augmentation in the duration of immobility in the FST and TST, as well as a surge in the locomotor activity of rats in OFT. Furthermore, castration exerts deleterious effects on the endogenous cellular levels of antioxidants. After castration, an elevation was observed in serum MDA levels, concomitant with a decline in SOD, GPx, and CAT levels. Rodents serve as optimal models for depression-like behaviors, and shifts in such behaviors have been observed after gonadectomy or ovariectomy. Research conducted on both humans and animals has yet to yield an unequivocal comprehension of the neural mechanisms and etiology of anxiety. By increasing testosterone levels through either endogenous factors or exogenous administration, symptoms of depression have been observed to diminish in individuals. Studies have demonstrated a close association between dysphoric mood and hypogonadism (Jung & Shin, 2017). Few investigations have evaluated the correlation between testosterone levels and depression. Moreover, studies have shown that reduced testosterone and dehydroepiandrosterone levels are linked to depression (Young et al., 2020). Imipramine, an inhibitor of norepinephrine reuptake, is commonly prescribed to patients due to its efficacy and affordability (Bangasser & Cuarenta, 2021). However, the side effects associated with imipramine have spurred escalating interest in the utilization of novel antidepressant agents that offer similar therapeutic properties whilst minimizing adverse effects (Silva Almodóvar et al., 2022).

Based on the current results, the administration of betaine in conjunction with castration reduced the duration of immobility in the FST and TST, as well as the locomotor activity of the rat in the OFT. Furthermore, betaine markedly attenuated serum MDA levels and augmented SOD, GPx, and CAT levels. Earlier reports have documented that betaine (30 and 100 mg/kg) reduced the duration of immobility in the FST. Furthermore, betaine at 30 mg/kg dose yielded results similar to fluoxetine (10 mg/kg) (Kim et al., 2013). Haramipour et al. (2021) reported that betaine (50 mg/kg) attenuated depressive behavior after ovariectomy in mice. Additionally, injection of betaine (30 and 100 mg/kg) reduced the duration of immobility in the FST in rats (Ohnishi et al., 2019). Antidepressant medications typically exert their effects through the serotonergic, adrenergic, and nitric oxide systems within the brain, and betaine has impeded nitric oxide production during oxidative stress. Furthermore, peripheral administration of betaine across the blood-brain barrier has elevated serotonin levels in the hypothalamus and hippocampus, functioning as a neurocognitive and neuroprotective mediator (Zhao et al., 2018).

Conclusion

In summary, the results of this investigation suggest that betaine, a natural antioxidant, exerts a favorable influence on ameliorating depressive behaviors induced by castration. Further research is warranted to elucidate the underlying mechanisms responsible for the observed outcomes.

Ethical Considerations

Compliance with ethical guidelines

There were no ethical considerations to be considered in this research.

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Authors' contributions

Data collection, writing the original draft: Jina Ghasemi Jalal; Supervisor, methodology, review and editing: Shahin Hassanpour.

Conflict of interest

The authors declared no conflict of interest.

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مقاله پژوهشی

اثرات بتائین در کاهش افسردگی ناشی از گنادکتومی در موش صحرایی

ژینا قاسمی جلال[1] ، *شاهین حسن پور[2]

۱. دانشکده دامپزشکی، واحد علوم و تحقیقات، دانشگاه آزاد اسلامی، تهران، ایران.
۲. بخش فیزیولوژی، گروه علوم پایه، دانشکده دامپزشکی، واحد علوم تحقیقات، دانشگاه آزاد اسلامی، تهران، ایران.

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چکیده

زمینه مطالعه: افسردگی یکی از اختلالات روانی عمده است که بر اساس اختلال خلقی طبقه بندی می شود و بتائین دارای فعالیت ضد درد در موش ها می‌باشد.

هدف: این مطالعه با هدف تعیین فعالیت ضد افسردگی بتائین در موش های صحرایی نر گنادکتومی شده انجام شد.

روش کار: ۲۰ سر موش صحرایی نر بالغ به چهار گروه آزمایشی تقسیم شدند. گروه ۱ بعنوان کنترل نگه داشته شد و در سایر گروه ها گنادکتومی انجام شد. گروه ۲ گروه شم بود که بدون درمان اخته شد. در گروه ایمی پرامین، موش‌ها اخته شدند و ایمی‌پرامین (۱۵ میلی‌گرم بر کیلوگرم) به مدت دو هفته تجویز شد. در گروه ۴، به دنبال اخته کردن، موش‌ها داخل صفاقی قرار گرفتند. بتائین (۳۰ میلی گرم بر کیلوگرم) به ترتیب به مدت دو هفته تزریق شد. سپس تست‌های ضد افسردگی با استفاده از تست شنای اجباری (FST)، تست تعلیق دم (TST) و تست میدان باز (OFT) انجام شد. در پایان مطالعه، از هر موش قلبی نمونه خون گرفته شد و مالون دی آلدئید (MDA)، سوپراکسید دیسموتاز (SOD)، گلوتاتیون پراکسیداز (GPx) و کاتالاز (CAT) سرم تعیین شد.

نتایج: بر اساس یافته‌ها، گنادکتومی زمان بی‌حرکتی در FST و TST و فعالیت موش‌ها را در OFT به‌طور معنی‌داری افزایش داد (P<۰/۰۵). تجویز بتائین با اخته‌سازی باعث کاهش معنی‌دار زمان تحرک در FST و TST و حرکت موش در OFT شد. گروه درمان نشده (P<۰/۰۵). اخته کردن سطح سرمی MDA را به طور معنی‌داری افزایش داد و سطوح GPx، SOD و CAT را نسبت به گروه کنترل کاهش داد (P<۰,۰۵). بتائین سطح سرمی MDA را به طور معنی‌داری کاهش داد و سطوح GPx، SOD و CAT را نسبت به گروه کنترل افزایش داد (P<۰/۰۵).

نتیجه‌گیری نهایی: این نتایج حاکی از آن است که بتائین به عنوان یک آنتی اکسیدان طبیعی، تأثیر مفیدی در کاهش رفتارهای افسردگی ناشی از اختگی دارد.

کلیدواژه‌ها: بتائین، گنادکتومی، افسردگی، موش صحرایی

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* **نویسنده مسئول:**
دکتر شاهین حسن پور
نشانی: تهران، دانشگاه آزاد اسلامی، واحد علوم تحقیقات، دانشکده دامپزشکی، گروه علوم پایه، بخش فیزیولوژی.
تلفن: ۴۴۸۶۸۵۶۰ (۲۱) ۹۸+
رایانامه: *hassanpour.shahin@gmail.com*

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