DOI:10.22059/IJVM.2023.367753.1005466 Iranian Journal of Veterinary Medicine Original Article

Online ISSN: 2252-0554

Effects on Betaine on Ameliorating Depression in Gonadectomized Male Rats

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Abstract

BACKGROUND: Depression is one of the major mental disorders categorized by impairment in mood and Betaine has anti-nociceptive activity in mice.

OBJECTIVES: This study aimed to determine the anti-depressive activity of betaine in gonadectomized male rats.

METHODS: Twenty adult male rats were allocated into four experimental groups. Group 1 was kept in control, while in the other groups, castration was done. Group 2 was the sham group had castration with no treatment. In the imipramine group, rats were castrated and administered the imipramine (15 mg/kg) for two weeks. In group 4, following castration, rats were i.p. injected with betaine (30 mg/kg) for two weeks, respectively. Then anti-depressive tests were done using a forced swimming test (FST), tail suspension test (TST), and open field test (OFT). At the end of the study, blood samples were taken from each cardiac mouse, and serum malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) were determined.

RESULTS: Based on the findings, castration significantly increased immobility time on FST and TST and the activity of the rat in 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 suggested betaine, as a natural antioxidant, has a beneficial effect on decreasing castration-induced depressive behaviors.

Keywords: Betaine, Castration, Depression, Rat

1. 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. The supplementation of betaine exhibits antioxidative and anti-inflammatory effects, effectively mitigating oxidative stress, nuclear factor-κB, cyclooxygenase-2, and inducible nitric oxide production (Tiwari & Hemalatha, 2022). Numerous studies have been conducted to evaluate the efficacy of betaine in relation to brain-related disorders. It has been demonstrated that betaine holds promise as a novel psychotherapeutic agent for schizophrenia (Ohnishi *et al.,* 2019). Furthermore, the administration of betaine (30 mg/kg) has been shown to alleviate depression induced by zinc oxide toxicity (Jeyhoonabadi *et al.,* 2022). Additionally, betaine exhibits anti-nociceptive activity in mice, and its administration has been found to reduce immobility time in the Forced Swim Test (FST), while also elevating serotonin levels in the hippocampus and hypothalamus of rats (Kim *et al.,* 2013).

Depression represents a prominent mental disorder characterized by mood impairment, thereby exerting an influence on the physiological functioning of the brain and altering emotional and cognitive processes (Zhang *et al.,* 2022). Hormonal fluctuations are closely associated with depression, exerting an impact on the hypothalamic-pituitary-adrenal axis. Cortisol and corticotrophin-releasing hormone levels are elevated in individuals suffering from depression.

Imipramine, a norepinephrine reuptake inhibitor, is commonly prescribed to patients due to its efficacy and cost-effectiveness (Bangasser & Cuarenta, 2021). Extensive literature substantiates the significant role of sex hormones in depressive behavior among both genders. Testosterone depletion has been found to be associated with depression, whereas androgen deprivation therapy has been shown to ameliorate depressive behavior (Peng *et al.,* 2022). Androgen receptors located in the hippocampus, amygdala, hypothalamus, and cerebral cortex play a crucial role in the pathogenesis of mood and depression. Testosterone has the ability to traverse the blood-brain barrier and is present in the aforementioned regions. Diminished androgen levels lead to reduced activity in the hippocampus and amygdala, thereby increasing the risk of depression, whereas testosterone supplementation has been found to improve this condition (Alwhaibi *et al.,* 2022). Gonadectomy-induced depression in rats results in a decrease in cellular antioxidant enzymes, such as SOD, CAT, GPx, and glutathione, in the bloodstream (Maheshwari *et al.,* 2022). Given the potential side effects associated with imipramine, there is a 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 existing report on its antidepressant effect specifically in relation to gonadectomy-induced behavior in male rats. Thus, the objective of this study is to investigate the depressant effect of betaine following castration in rats.

2. Material and Methods

Animals

Twenty adult male Wistar rats weighing between 200 and 250 grams were assigned to four experimental groups, with each group consisting of five rats (n=5). The rats were housed in standard plastic cages under laboratory conditions and provided with unrestricted access to food and water. Once they 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 in order to access the abdominal cavity. For castrations, the blood supply to each testis was clamped using locking forceps, followed by the ligation of the testes with sterile sutures and their excision with a scalpel. The muscle and skin layers were subsequently sutured, and wound clips were applied over the incision for a duration of eight days to facilitate healing. Additionally, a meloxicam injection of 10 mg/kg was administered 24 hours after the 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, the rats were divided into four experimental groups (n=5). The surgeries performed on the control group were identical to those in the other groups, except that the testes were not clamped, ligated, or excised. The Sham group underwent castration without any further treatment. In the imipramine group, the rats were castrated and subsequently received a dosage of imipramine (15 mg/kg) for a duration of two weeks. In group 4, the rats were castrated and then administered betaine (30 mg/kg) through intraperitoneal injection for two weeks (Ueno *et al.,* 2022).

Forced Swimming Test

The forced swimming test involved placing each rat individually in a glass cylinder filled with water maintained at a temperature of 23 ± 2°C. The rats were required to swim for a total of six minutes, with the first two minutes serving as an adaptation period. The duration of immobility was recorded during the final four minutes of the session (Ueno *et al.,* 2022).

Tail Suspension Test

In the tail suspension test, the rats were suspended by 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 a four-minute period (Cryan *et al.,* 2005).

Open Field Test

The locomotor behavior of the rats was evaluated using the 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

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

Statistical Analysis

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

3. Results

The behavioral changes in castrated rats resulting from the administration of betaine are presented in Figure 1-3. As observed in Figure 1, castration had a significant effect on increasing the immobility time in castrated rats compared to the control group during the FST test (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). The 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 findings depicted in Figure 2, castration had a significant effect on increasing 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 resulted in a significant reduction in mobility time compared to the untreated group (P<0.05).

According to the data presented in Figure 3, castration had a significant impact on diminishing the activity of the rats during the OFT test 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 control group (P<0.05). The administration of betaine significantly increased the movement of the rats during the OFT test in the untreated group (P<0.05).

The antioxidant activity of betaine following castration is illustrated in Figures 4-7. As depicted in Figure 4, castration had a significant effect on increasing the serum MDA levels compared to the control group ($P<0.05$). Pretreatment with imipramine led to a significant decrease in serum MDA levels compared to control rats ($P<0.05$). Betaine administration resulted in a significant decrease in 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 castrated rats compared to the control group ($P<0.05$). Pretreatment with imipramine led to a significant increase in serum SOD levels compared to control rats ($P<0.05$). Betaine administration resulted in a significant increase in serum SOD levels compared to the control group ($P<0.05$).

As presented in Figure 6, serum GPx levels significantly decreased in castrated rats compared to the control group ($P<0.05$). Pretreatment with imipramine led to a significant increase in serum GPx levels compared to control rats ($P<0.05$). The administration of betaine resulted in a significant increase in serum GPx levels compared to the control group ($P<0.05$).

In this study, it was observed that serum CAT levels significantly decreased in rats following castration ($P<0.05$). Pretreatment with imipramine led to a significant increase in 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).

4. Discussion

Based on the principal findings of the present investigation, castration elicited an augmentation in the duration of immobility on the FST and TST, as well as a surge in the locomotor activity of the rat on the OFT. Furthermore, castration exerted deleterious effects on endogenous cellular

levels of antioxidants. Post-castration, there was an elevation in serum MDA levels, concomitant with a decline in SOD, GPx, and CAT levels. Rodents serve as optimal models for depressive-like behaviors, and shifts in such behaviors have been observed subsequent to 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 the levels of testosterone 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). Scarce investigations have evaluated the correlation between testosterone and depression. Moreover, studies have evinced that reduced levels of testosterone and dehydroepiandrosterone 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 an escalating interest in the utilization of novel antidepressant agents that offer similar therapeutic properties whilst minimizing adverse effects (Silva Almodóvar *et al.,* 2022).

Ascertained from the current findings, the administration of betaine in conjunction with castration served to diminish the duration of immobility on the FST and TST, as well as reduce the locomotor activity of the rat on the OFT. Furthermore, betaine markedly attenuated serum MDA levels and concurrently augmented SOD, GPx, and CAT levels. Earlier reports have documented that betaine (at doses of 30 and 100 mg/kg) reduced the duration of immobility in the FST. Furthermore, betaine at a dose of 30 mg/kg yielded analogous results to fluoxetine (at a dose of 10 mg/kg) (Kim *et al.,* 2013). Haramipour *et al.,* (2021) reported that betaine (at a dose

of 50 mg/kg) attenuated depressive behavior subsequent to ovariectomy in mice. Additionally, injection of betaine (at doses of 30 and 100 mg/kg) resulted in a reduction in the duration of immobility in the FST in rats (Ohnishi *et al.,* 2019). Antidepressant medications typically exert their effects through serotonergic, adrenergic, and nitric oxide systems within the brain, and betaine has been shown to impede nitric oxide production during oxidative stress. Furthermore, peripheral administration of betaine across the blood-brain barrier has been found to elevate serotonin levels in the hypothalamus and hippocampus, thereby functioning as a neurocognitive and neuroprotective mediator (Zhao *et al.,* 2018).

5. Conclusions

In summary, the findings of this investigation suggest that betaine, as a natural antioxidant, exerts a favorable influence in ameliorating depressive behaviors induced by castration. Further research endeavors are warranted to elucidate the underlying mechanisms responsible for these observed outcomes.

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اثرات بتائین در کاهش افسردگی ناشی از گنادکتومی در موش صحرایی

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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 می باشد.

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

روش کار: 20 سر موش صحرایی نر بالغ به چهار گروه آزمایشی تقسیم شدند. گروه 1 بعنوان کنترل نگه داشته شد و در سایر گروه ها گنادکتومی انجام شد. گروه 2 گروه شم بود که بدون درمان اخته شد. در گروه ایمی پرامین، موش‌ها اخته شدند و ایمی‌پرامین (15 میلی‌گرم بر کیلوگرم) به مدت دو هفته تجویز شد. در گروه 4، به دنبال اخته کردن، موش‌ها داخل صفاقی قرار گرفتند. بتائین (30 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 ($P<0.05$). تجویز بتائین با اختەسازی باعث کاهش معنی‌دار زمان تحرک در FST و TST و حرکت موش در OFT شد. گروه درمان نشده ($P<0.05$). اختە کردن سطح سرمی MDA را به طور معنی‌داری افزایش داد و سطوح SOD، GPx و CAT را نسبت به گروه کنترل کاهش داد ($P<0.05$). بتائین سطح سرمی MDA را به طور معنی‌داری کاهش داد و سطوح SOD، GPx و CAT را نسبت به گروه کنترل افزایش داد ($P<0.05$).

نتیجه گیری 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.

کلمات کلیدی: بتائین، گنادکتومی، افسردگی، موش صحرایی