http://jbcp.shahed.ac.ir 10.22070/jbcp.2023.17619.1170

Open Access Research Article

Journal of Basic and Clinical Pathophysiology
Print ISSN: 2322-1895, **Online ISSN:** 2345-4334
Vol. 11, No. 1, June 2023

A Semiannual Publication of
Shahed University Press

Anti-depressant effect of α-pinene on gonadectomized-related behavior in male rats

Shahin Hassanpour [1*], Arghavan Bahrami [2]

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

Abstract

Objective: This study aimed to determine anti-depressant effect of α-pinene on gonadectomized-related behavior in male rats.

Materials and Methods: Thirty adult male Wistar rats were assigned to 5 experimental groups. In the control group, surgeries were identical but testes were not clamped, ligated or excised. In the sham control, surgeries were identical with no treatment. In the imipramine group, rats were administered with imipramine (15 mg/kg) followed by castration for 2 weeks. In groups 4 and 5, following castration, rats were i.p. injected with α-pinene (0.5 and 1 mg/kg) for 2 weeks, respectively. Following recovery, forced swimming test (FST), tail suspension test (TST), and open field test (OFT) were performed and serum levels of malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) activity were determined.

Results: According to the data, immobility time significantly increased following castration compared to control group ($p<0.05$). Administration of the imipramine significantly decreased immobility time compared to control group ($p<0.05$). Treatment with α-pinene (0.5 and 1 mg/kg) significantly decreased immobility time in comparison with sham control group ($p<0.05$). Number of crossings in the OFT significantly diminished following castration and treatment with α-pinene (0.5 and 1 mg/kg) increased number of crossings in the OFT compared to sham control group ($P<0.05$). Serum MDA significantly increased while SOD, GPX and CAT decreased following castration compared to the control group ($p<0.05$). Administration of the α-pinene (0.5 and 1 mg/kg) significantly improved serum MDA, SOD, GPX and CAT production compared to the sham rat ($p<0.05$).

Conclusion: These results suggested that α-pinene has anti-depressant and antioxidant effects in gonadectomized-related behavior in male rats

Keywords: α-pinene, Anti-depressant, Gonadectomy

1. Introduction

Depression and anxiety disorders are the most common emotional disorders that are associated with biochemical, cognitive, behavioral, and psychological changes in the brain in both men and women (1). Depression is associated with hormonal fluctuations, which influences hypothalamic-pituitary-adrenal axis. Depression is associated with elevated cortisol and corticotrophin-releasing hormone levels. Imipramine, a norepinephrine reuptake inhibitor, is typically prescribed to patients due to its effectiveness and lower price (2). Due to side effects of imipramine, there is growing interest to use new antidepressants with similar medicinal ability and lower side effects (3). Testosterone replacement in castrated men improves depressive behavior but on conditions such as prostate cancer is not a good choice for depression treatment. Also, cellular antioxidant enzymes such as SOD, CAT, GPx, and glutathione are antioxidants that are decreased in the blood of rats due to the orchiectomy-induced depression (4). Thus, there is

Submitted: 21 July 2022, **Revised:** 21 November 2022, **Accepted:** 16 December 2022
***Corresponding Author:** Shahin Hassanpour **E-mail:** s.hassanpour@srbiau.ac.ir; hassanpour.shahin@gmail.com

growing interest for using herbal medications with lower side effects (5).

α-pinene ($C_{10}H_{16}$) is a nature terpenoid with fungicidal, antibacterial, anticancer and anti-nociceptive property (5). It has been reported that α-pinene has neuroprotective activity and has potential against neurodegenerative diseases, like Alzheimer's and Parkinson's (6). α-pinene has antioxidant properties by improving brain-derived neurotrophic factor (BDNF) and oxidative imbalance (7). α-pinene administration inhibits reactive oxygen species (ROS) and MDA production and improves catalase, SOD, GPx and glutathione reductase levels (8). α-pinene decreases lipid peroxidation in the rat with Parkinson's disease (9). Also, α-pinene has low molecular weight which can cross blood-brain barrier (5).

Based on role of the of the α-pinene in the nervous system and lack of information on its possible anti-depressant effect, this study aimed to determine anti-depressant effect of α-pinene on gonadectomized-related behavior in male rats.

2. Materials and Methods

2.1. Animals

Thirty adult male Wistar rats (200–250 g) were 5 assigned to experimental groups (n=6/group). Rats were kept in standard plastic cages at laboratory conditions (temperature of 22 ± 2°C and 12 h light/dark cycle). Food and tap water were available ad libitum. One week after adaptation of the animals to new laboratory condition, surgery procedure was done. The incision area was shaved and scrubbed with ethanol and betadine. Ophthalmic ointment was placed over the eyes to prevent drying. A 1 cm incision was made with a scalpel in the lower abdomen across the midline to access the abdominal cavity. For castration, the blood supply to each testis was clamped with locking forceps, after which the testes were ligated with sterile sutures and excised with a scalpel. The muscle and skin layers were then sutured, and wound clips were placed over the incision for 8 days to allow the incision to heal. An additional injection of 10 mg/kg meloxicam was given 24 hours after surgery (10). Rats were allowed to recover and were post-surgically monitored for a week for signs of discomfort or distress (11).

2.2. Experimental design and treatments

Following recovery, the rats were divided into five experimental groups (n=6/group). The control group had surgeries that were identical but testes were not clamped, ligated or excised. In the sham control, surgeries were identical with no treatment. In the imipramine group, rats were administered with imipramine (15 mg/kg) followed by castration for 2 weeks. In groups 4 and 5, following castration, rats were i.p. injected with α-pinene (0.5 and 1 mg/kg) (98.0%>purity, Sigma Chemical Co., St Louis, MO, USA) for 2 weeks, respectively.

2.3. Behavioral tests

2.3.1. Forced swimming test

The method followed was that described by Boissy (12). Swimming sessions were conducted by placing the rat in individual glass cylinders (height = 50 cm, diameter = 30 cm) containing 30 cm of water at 23 ± 2°C. During the session, rats were forced to swim for 6 minutes and the duration of immobility was measured for 4 minutes (13).

2.3.2. Tail suspension test

Animals were suspended on a horizontal beam (height 33 cm) using adhesive tape wrapped around the tip of the tail. The time spent immobile was recorded during 6 minutes (14).

2.3.3. Open field test

The locomotors behavior was accessed through an OFT. The test was done using a 45×45×30 cm^3 cage while the floor of this area was divided into 3×3 cm^2 equally-sized squares. Each rat was put in the center of the open field box. After two minutes, the number of squares that all four feet traveled over them were documented for 4 minutes. Between tests, the field was cleaned with 70% ethanol to remove odor bias (15).

2.4. Antioxidant assay

After performing behavioral tests, blood samples were taken from each the heart and serum MDA, SOD, GPx, and CAT were determined using Zell Bio GmbH (Germany) assay kits.

3. Results

Effects of imipramine and α-pinene on FST in gonadectomized rats is presented in figure 1. As seen, immobility time significantly increased in sham control rats compared to the control group ($p<0.05$). Administration of the imipramine significantly decreased immobility time compared to the control group ($p<0.05$). Treatment with α-pinene (0.5 and 1 mg/kg) significantly decreased immobility time in comparison to the sham control group ($p<0.05$).

As shown in figure 2, immobility time in TST significantly increased following castration compared to the control group ($p<0.05$). Treatment with imipramine significantly decreased immobility time in comparison to the sham control group ($p<0.05$). Administration of the α-pinene (0.5 and 1 mg/kg) significantly decreased immobility time in comparison to the sham control group ($p<0.05$) .

According to figure 3, number of crossings in the OFT significantly diminished in the sham control rat compared to the control group ($p<0.05$). Treatment

with imipramine significantly increased number of crossings in the OFT in comparison to the control group ($p<0.05$). Administration of α-pinene (0.5 and 1 mg/kg) significantly increased number of crossings in the OFT in comparison to the sham rat ($p<0.05$).

Effects of imipramine and α-pinene on serum antioxidant levels in gonadectomized rats is presented in figures 4-7. According to figure 4, serum MDA production significantly increased following castration compared to the control group ($p<0.05$). Administration of imipramine significantly decreased serum MDA production compared to the control group ($p<0.05$). Administration of α-pinene (0.5 and 1 mg/kg) significantly diminished serum MDA production compared to the sham rat ($p<0.05$).

As seen in figure 5, serum SOD levels significantly decreased following castration compared to the control group ($p<0.05$). Administration of α -pinene (0.5 and 1 mg/kg) significantly improved serum SOD levels in comparison to the sham control group ($p<0.05$).

In this study, serum GPx levels significantly decreased in the control sham rats in comparison to the control group ($p<0.05$). Administration of imipramine significantly increased GPx compared to the control group ($p<0.05$). α -pinene (0.5 and 1 mg/kg) significantly improved serum GPx levels compared to the sham group ($p<0.05$) (figure 6).

As observed, serum CAT level decreased following castration compared to the control group ($p<0.05$). Administration of imipramine significantly increased CAT compared to the control group ($p<0.05$). Treatment with α -pinene (0.5 and 1 mg/kg) significantly improved serum CAT level compared to the sham group ($p<0.05$) (figure 7).

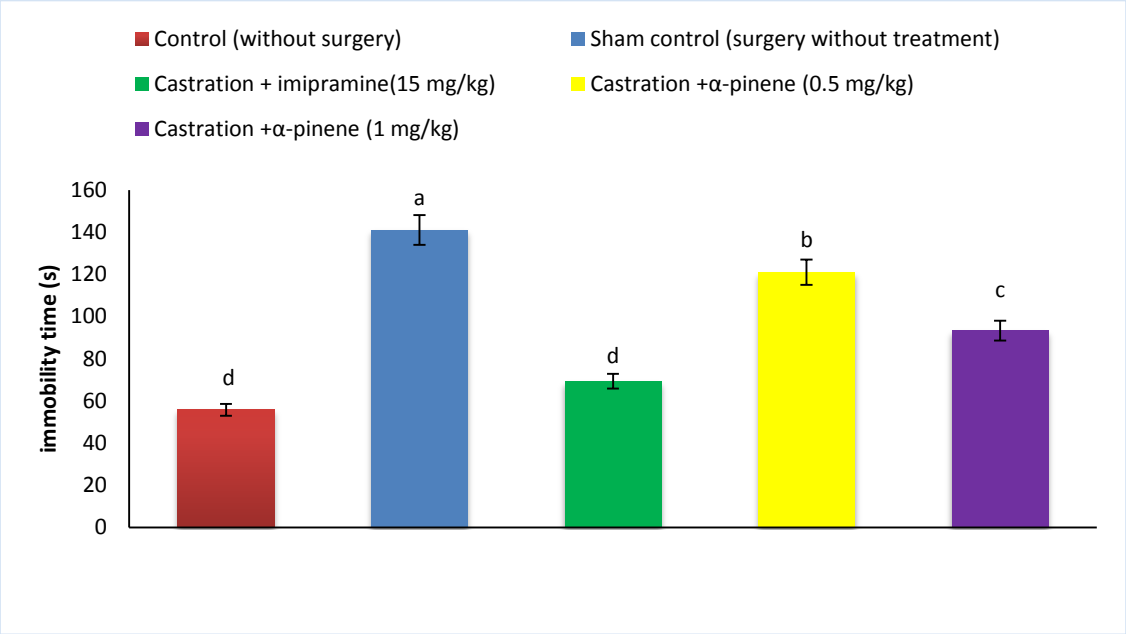

Figure 1. Effects of imipramine (15 mg/kg), α-pinene (0.5 and 1 mg/kg) on FST (forced swimming test) in gonadectomized rats. Different letters (a-d) indicate significant differences between treatments ($P<0.05$).

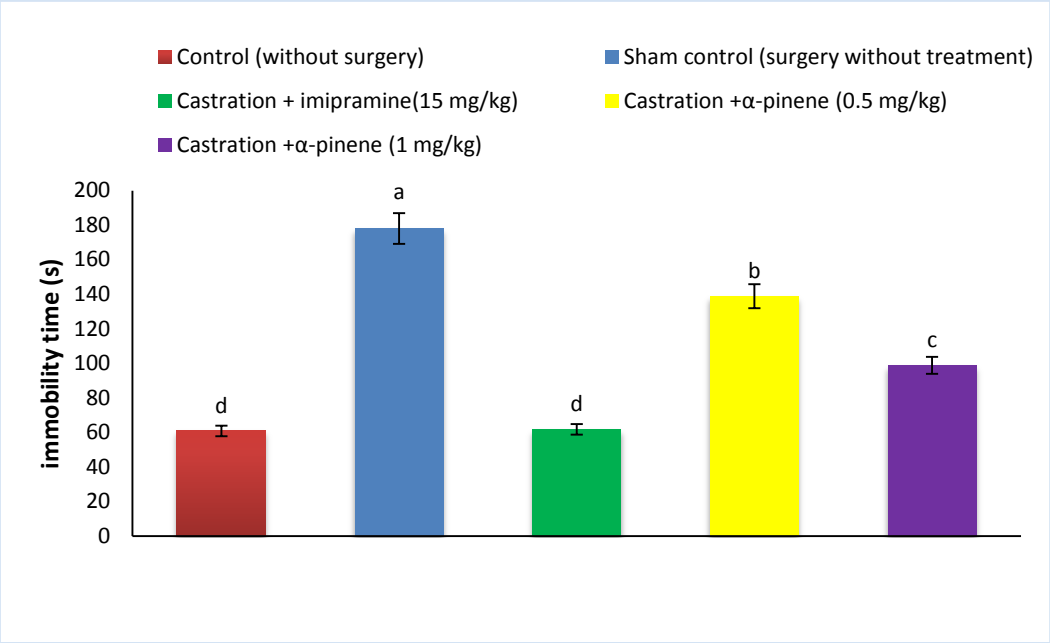

Figure 2. Effects of imipramine (15 mg/kg), *α*-pinene (0.5 and 1 mg/kg) on TST (tail suspension test) in gonadectomized rats. Different letters (a-d) indicate significant differences between treatments (P<0.05).

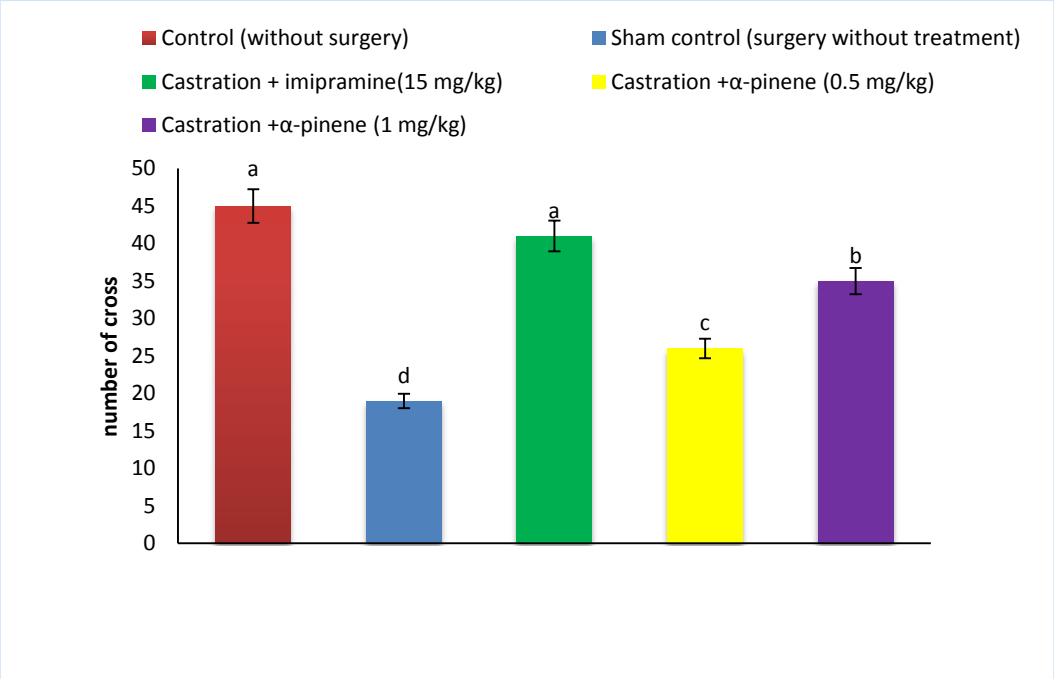

Figure 3. Effects of imipramine (15 mg/kg), *α*-pinene (0.5 and 1 mg/kg) on OFT (open field test) in gonadectomized rats. Different letters (a-d) indicate significant differences between treatments (P<0.05).

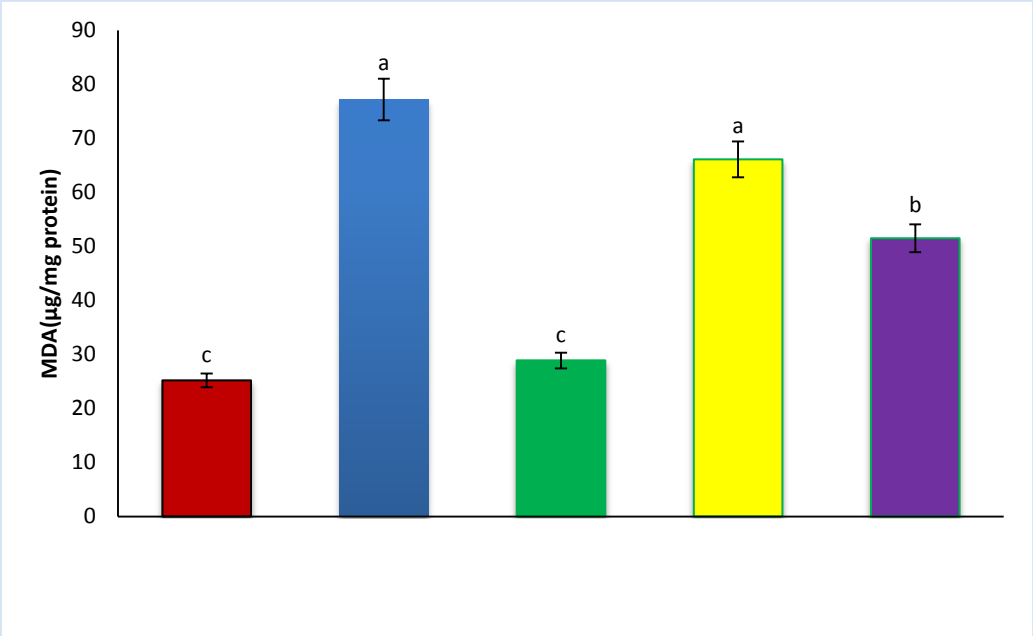

Figure 4. Effects of imipramine (15 mg/kg), α-pinene (0.5 and 1 mg/kg) on MDA (malondialdehyde) in gonadectomized rats. Different letters (a-d) indicate significant differences between treatments (P<0.05).

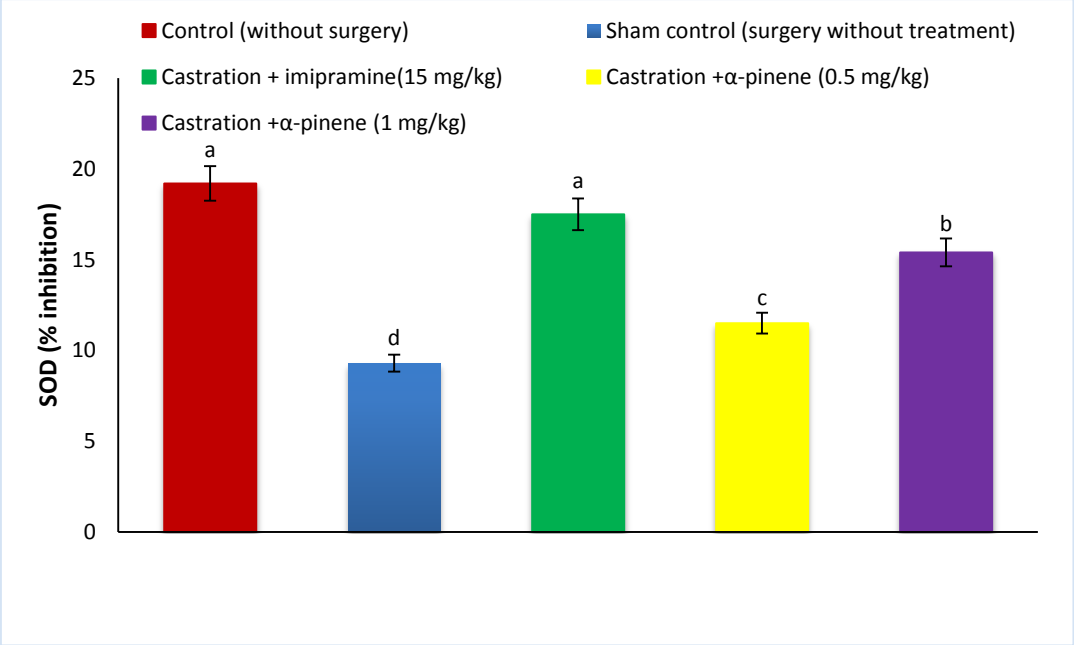

Figure 5. Effects of imipramine (15 mg/kg), α-pinene (0.5 and 1 mg/kg) on SOD (superoxide dismutase) in gonadectomized rats. Different letters (a-d) indicate significant differences between treatments (P<0.05).

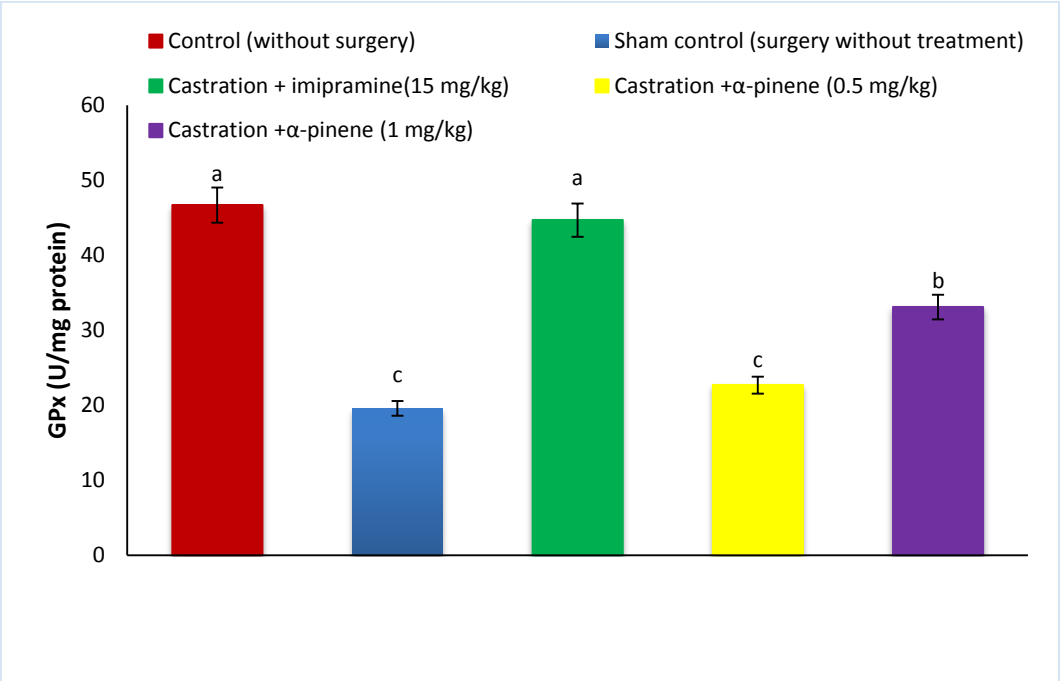

Figure 6. Effects of imipramine (15 mg/kg), *α*-pinene (0.5 and 1 mg/kg) on GPx (glutathione peroxidase) in gonadectomized rats. Different letters (a-d) indicate significant differences between treatments (P<0.05).

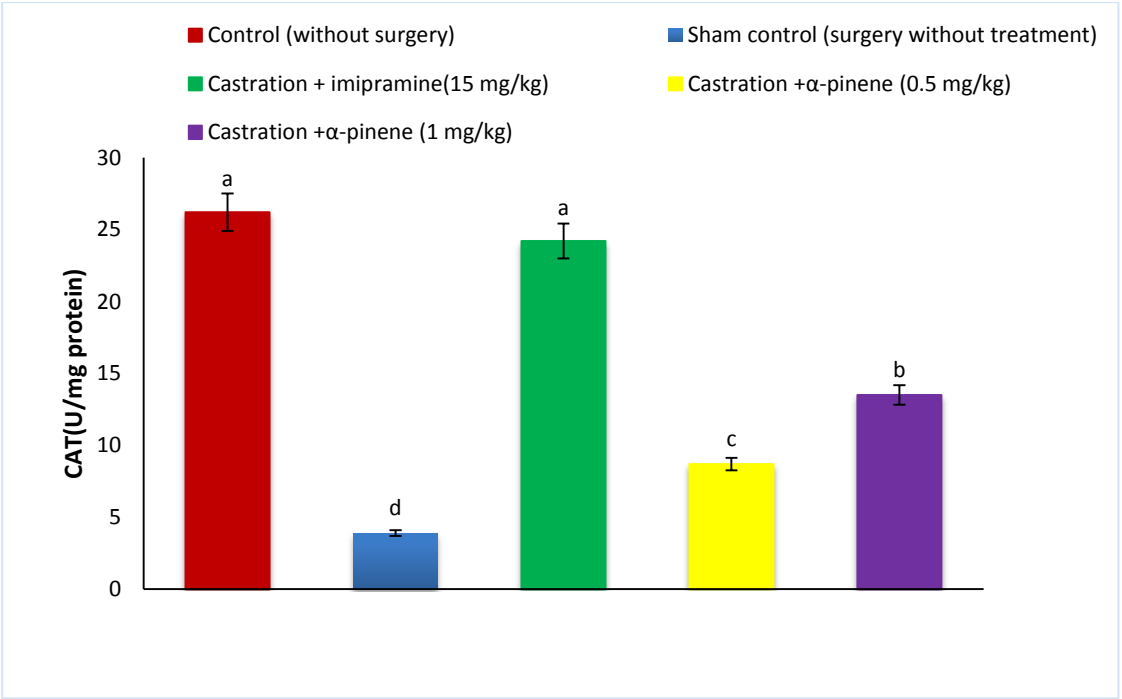

Figure 7. Effects of imipramine (15 mg/kg), *α*-pinene (0.5 and 1 mg/kg) on CAT (catalase) in gonadectomized rats. Different letters (a-d) indicate significant differences between treatments (P<0.05).

4. Discussion

Depressive disorders are the most common mental disorders associated with biochemical, cognitive, behavioral, and psychological changes. These disorders are observed differently among both sexes, but these depression disorders in men are about half compared to women. Currently, research on humans and animals has not yet provided a clear understanding of the neural mechanisms and causes of depression (16). A close relationship between depressed mood and hypogonadism is reported. Testosterone levels are deficient in men with severe and treatment-resistant depression and depressed older men (17). As observed in the current study, antioxidant and anti-depressive activity observed for α-pinene. Recently, anti-anxiety effect reported for α-pinene in comparison with Diazepam in rats. Saeedi and Rafiei-Rad (18) reported that α-pinene (2 and 4 mg/kg) affects time spent in the open arm as well as decreases serum MDA and increases GPx activity which was in agreement to this report. Administration of α-pinene (50 mg/kg, intraperitoneally) for 14 consecutive days strengthens the antioxidant system and prevents neuroinflammation in the hippocampus of rats after intrahippocampal injection of beta-amyloid (Aβ)1-42 (19).

This is well documented that MDA level and antioxidant enzyme activities (SOD, CAT and GPx) are important biomarker in anxiety and affective disorders (20). α-pinene stimulates postsynaptic chloride flow linked to GABAA receptors (6). Neuroprotective activity is reported for α-pinene. Yang et al (6) reported that oral administration of α-pinene (200 mg/kg) prior to pentobarbital decreases sleep latency. α-pinene increases BDNF gene expression in the hippocampus, the main factor for cell survival and neurogenesis (21). α-pinene prevents ROS generation and lipid peroxidation and damage (22). α-pinene has low molecular weight which can cross blood-brain barrier (5). Also, α-pinene increases CAT, SOD and GPx in H2O2-sensitized oxidative stress by reducing apoptosis and defending the nervous system. Based on our findings, it seems that α-pinene has anti-depressant and antioxidant effects in gonadectomized-related behavior in male rats. Despite researches that have been done on neuroprotective role of α-pinene, but direct mechanisms of its action are not fully known. It seems that α-pinene can potentiate by binding to the position of benzodiazepines in GABAA receptors and may exert its anti-anxiety effect with antioxidant properties (18). Further researches needed to determine mechanisms of action for observed results.

These results suggested that α-pinene has anti-depressant and antioxidant effects in gonadectomized-related behavior in male rats.

References

1. Angermann CE, Ertl G. Depression, Anxiety, and Cognitive Impairment. Current Heart Failure Reports. 2018;15(6):398-410.
2. Bangasser DA, Cuarenta A. Sex differences in anxiety and depression: circuits and mechanisms. Nature Reviews Neuroscience. 2021;22(11):674-84.
3. Silva Almodóvar A, Nguyen D, Nahata MC. Evidence Needed for Efficacy of Antidepressant Medications Among Patients With Rheumatoid Arthritis. Annals of Pharmacotherapy. 2022:10600280211062271.
4. Maheshwari N, Khan AA, Ali A, Mahmood R. Oral administration of pentachlorophenol impairs antioxidant system, inhibits enzymes of brush border membrane, causes DNA damage and histological changes in rat intestine. Toxicology Research. 2022.
5. Ghosh S, Kumar A, Sachan N, Chandra P. Anxiolytic and antidepressant-like effects of essential oil from the fruits of Piper nigrum Linn.(Black pepper) in mice: involvement of serotonergic but not GABAergic transmission system. Heliyon. 2021 Apr 1;7(4):e06884.
6. Yang H, Woo J, Pae AN, Um MY, Cho NC, Park KD, Yoon M, Kim J, Lee CJ, Cho S. α-Pinene, a major constituent of pine tree oils, enhances non-rapid eye movement sleep in mice through GABAA-benzodiazepine receptors. Molecular pharmacology. 2016 Nov 1;90(5):530-9.
7. Ueno, H., Shimada, A., Suemitsu, S., Murakami, S., Kitamura, N., Wani, K., Matsumoto, Y., Okamoto, M., & Ishihara, T. (2020). Alpha-pinene and dizocilpine (MK-801) attenuate kindling development and astrocytosis in an experimental mouse model of epilepsy. IBRO Reports, 9,102-14.
8. Türkez H, Aydın E. In vitro assessment of cytogenetic and oxidative effects of α-pinene. Toxicology and Industrial Health 2016; 32(1),168-76
9. Goudarzi S, Rafieirad M. Evaluating the effect of α-pinene on motor activity, avoidance memory and lipid peroxidation in animal model of Parkinson disease in adult male rats. Research Journal of Pharmacognosy 2017; 4(2):53-63.
10. Huh JS, Chung BH, Hong CH, Ryu JK, Kim JH, Han WK, et al. The effects of testosterone replacement on penile structure and erectile function after long-term castration in adult male rats. International journal of impotence research. 2018;30(3):122-8.

11. Boivin JR, Piekarski DJ, Wahlberg JK, Wilbrecht L. Age, sex, and gonadal hormones differently influence anxiety-and depression-related behavior during puberty in mice. Psychoneuroendocrinology. 2017 Nov 1;85:78-87.

12. Boissy A. Fear and fearfulness in animals. The quarterly review of biology. 1995 Jun 1;70(2):165-91.

13. Ueno H, Takahashi Y, Murakami S, Wani K, Matsumoto Y, Okamoto M, et al. Effect of simultaneous testing of two mice in the tail suspension test and forced swim test. Scientific Reports. 2022;12(1):1-14.

14. Cryan JF, Mombereau C, Vassout A. The tail suspension test as a model for assessing antidepressant activity: review of pharmacological and genetic studies in mice. Neuroscience & Biobehavioral Reviews. 2005 Jan 1;29(4-5):571-625.

15. Takeda H, Tsuji M, Matsumiya T. Changes in head-dipping behavior in the hole-board test reflect the anxiogenic and/or anxiolytic state in mice. European journal of pharmacology. 1998;350(1):21-9.

16. Jung HJ, Shin HS. Effect of Testosterone Replacement Therapy on Cognitive Performance and Depression in Men with Testosterone Deficiency Syndrome. wjmh. 2017;34(3):194-9.

17. Drobisz D, Damborská A. Deep brain stimulation targets for treating depression. Behavioural brain research. 2019;359:266-73.

18. Saeedipour S, Rafieirad M. Anti-anxiety effect of Alpha-pinene in comparison with Diazepam in adult male rats. Feyz 2020; 24 (3) :253-245

19. Khan-Mohammadi-Khorrami MK, Asle-Rousta M, Rahnema M, Amini R. Neuroprotective effect of alpha-pinene is mediated by suppression of the TNF-α/NF-κB pathway in Alzheimer's disease rat model. Journal of Biochemical and Molecular Toxicology. 2022 May;36(5):e23006.

20. Kuloglu M, Atmaca M, Tezcan E, Gecici Ö, Tunckol H, Ustundag B. Antioxidant enzyme activities and malondialdehyde levels in patients with obsessive-compulsive disorder. Neuropsychobiology. 2002;46(1):27-32.

21. Masuo Y, Satou T, Takemoto H, Koike K. Smell and Stress Response in the Brain: Review of the Connection between Chemistry and Neuropharmacology. Molecules. 2021 Jan;26(9):2571.

22. Karthikeyan R, Kanimozhi G, Prasad NR, Agilan B, Ganesan M, Srithar G. Alpha pinene modulates UVA-induced oxidative stress, DNA damage and apoptosis in human skin epidermal keratinocytes. Life sciences. 2018 Nov 1;212:150-8.