Protective effects of pistachio oil (Pistacia vera) on follicle development in C57BL/6J mice model undergoing chronic unpredictable mild stress

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Information	Abstract
 Corresponding Author: *Morteza Anvari* *Email:* moanvari@yahoo.com *Tel:* +98-9131528128	**Background:** Women are sensitized to stress-related psychiatric disorders, such as depression and anxiety. Several studies have shown that follicular development and ovarian reserve are reduced after chronic stress. Objectives: In this experimental study, the effect of oral consumption of pistachio oil was evaluated on mice models undergoing chronic unprintable mild stress**.** **Material and Methods:** 6–8-week-old female C57BL/6J mice were randomly divided into 4 groups: one control and three experimental groups (n= 8). Animals in experimental groups (I, II, and III groups) were exposed to a variety of chronic unpredictable stress for 4 weeks. Then, the mice in I and II groups were fed orally 1 and 4 ml/kg/day pistachio oil for 4 weeks, respectively. Animals in the III and control groups received tab water. Forced swimming test (FST), sucrose preference test (SPT), and tail suspension test (TST) were performed to evaluate behavioral despair and hedonic level in mice. Their ovaries were analyzed for the number and diameter of primordial, primary, secondary, and antral follicles. **Results:** FST showed depressive-like behavior to be increased in stress groups compared with the control. According to SPT, greater data deviation was observed in the control mice compared to the experimental groups (P = 0.036). Follicle cell numbers showed a significant decrease in the I and III groups compared to the control (p = 0.034). In addition, there was a remarkable decreasing trend in the diameter of secondary and antral follicles in the I and III groups (p = 0.039). **Conclusion:** This study demonstrates that pistachio has protective impacts on unpredictable chronic mild stress in the mice model. Therefore, pistachio could be a potential medical supplement for improving follicular development and ovarian reserve**.**

1. Introduction

Women in the fertility age are susceptible to stress-related psychiatric disorders, such as depression and anxiety [1] . The prevalence of these disorders in women is approximately 2 to 3 times higher than in men [2]. Studies have shown important relationships between sex hormones and stress [3, 4] . Some researchers have reported that the changes in reproductive hormone levels are associated with the pathophysiology of stress and depression disorders [5, 6] . Moreover, chronic stress affects specifically women's reproductive functions, leading to ovulation disorders and infertility; for example, repeated stress can damage oocyte maturation. [7] . Chronic stress may also alter follicular growth and lead to a decrease in antral follicles by activating the sympathetic nerves in the ovary[8]. In animals, different stress models have been developed to better clarify stress. Unpredictable chronic mild stress (UCMS) is defined as exposure to some mild stress in experimental animals[9]. Studies have shown that UCMS is a condition in rats similar to human depression [10, 11]. Xu et al. (2020) report that unpredictable chronic stress not only prevents the development of secondary follicles and antral follicles in mice but also increases follicular atresia [12].

The follicles are the basic units of the ovary, being able to produce oocytes and secrete steroid hormones. In mammalian ovaries, primary follicles are arranged during the embryonic stage. Existing follicles may eventually lead to ovulation or atresia. Therefore, the growth and development of follicles play a vital role in female reproduction. This process is not only influenced by various growth hormones, endocrine, exocrine, and paracrine factors, such as brain-derived neurotrophic factor (BDNF) but

also is regulated by a variety of signaling pathways [13] . Although BDNF is primarily produced by the brain and affects the growth and survival of nerve cells, it may be associated with depression; also, it is expressed in the ovaries [14] . BDNF activates its downstream protein kinase signaling pathway, rapamycin (PI3K-AKT-mTOR) phosphoinositide-3 kinase, after binding to its specific receptor. The PI3K-AKT-mTOR signaling pathway plays a key role in cell proliferation, differentiation, apoptosis, as well as activation of primary follicles and oocyte meiosis [15] . Furthermore, Liu (2019) has reported PI3K-AKT-mTOR signaling pathway activated by BDNF in polycystic ovary syndrome (PCOS) and premature ovarian failure (POF) [16].

Pistachio nuts (Pistacia vera) are unique sources of various compounds, such as unsaturated fatty acids, β-carotene, α-tocopherol, flavonoids, and lutein [17]. Further, pistachios have been shown to have antioxidant and anti-inflammatory factors [18]. Unsaturated essential fatty acids in pistachio oil (oleic, linoleic, and linolenic acid) can prevent cardiovascular disease and elevated cholesterol in the human body. The oil contains mineral elements, including selenium, zinc, calcium, potassium, iron, and magnesium [19]. According to some studies, compounds in pistachio oil inhibit the production of nitric oxide (NO). Since this compound can inhibit steroidogenesis, pistachio and its oil is used as herbal medicine to treat sexual-related diseases, such as sexual impotency [20, 21]. There is much evidence that shows the effect of phytosterols on the sexual cycle and sex hormones [22]. Beta-sitosterol is one of the main phytosterol in the nuts. Further, it has been

reported that pistachios have high levels of beta-sitosterol, campesterol, and stigmasterol [23].

Since the effect of pistachio oil (pistacia vera) on the ovary is not clear, this study aims to investigate its effect on the development of follicles in the stress model on female mice.

2. Materials and methods

2.1. Plant material collection and oil extraction

Fresh fruits of Akbari pistachio were approved by an expert in the Department of Botany, Rafsanjan Valiasr University, Iran (genetic code: M30). Oil extraction was conducted after drying and using the cold press method, and the filtered oil (centrifuged at 5000 rpm for 10 min) was stored in the refrigerator [24].

2.2. Animals and ethics

In this experimental study, thirty-two 6-8weeks old female C57BL/6J mice (28.32±3.2 g) were obtained from Yazd Reproduction Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran. The animals were housed in individual cages in a climate-controlled room and under conditions of 12 h light/dark and 50 % relative humidity. Animal treatment methods were reviewed and approved by the Ethics Committee of Yazd Reproduction Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

The mice were randomly assigned to control or experimental groups (I, II, and III groups). Animals in the control group received water intragastrically (n = 8). Experimental groups (I, II, and III) were exposed to a variety of chronic unpredictable stress for 4 weeks (n=eight in each group) [25]. To have the stress-induced model, animals were exposed to sequential stressors, including electric foot shock (20 secs, 0.6 mA, 1-s duration, average 1 shock/10s), food deprivation (24 h), crowding (10 hours, 5–6 mice cage), water deprivation (24 hours), wet bedding (15 hours), an elevated open platform (2 hours, 10 cm × 10 cm, 160 cm in height), and restraint stress)2 h) [25] . After 4 weeks, stress-induced mice in I and II groups were fed orally 1 and 4 ml/kg/day pistachio oil for 4 weeks, respectively. Animals in the III group received tab water as a vehicle gavage after sequential stressors.

2.3. Behavioral tests

At the end of the treatment, forced swimming test (FST), sucrose preference test (SPT), and tail suspension test (TST) were performed to evaluate behavioral despair and anhedonia level in mice, respectively [26]. For FST, the mice were forced to swim in a water cylinder (21 cm diameter × 46 cm high containing 30 cm depth of water 25 °C), and the immobility time was assessed using a SMART video-tracking system in each mouse (Panlab, Spain). For SPT, there were two bottles of 1.5% sucrose for 24 h, one of which was replaced with water for the next 24 h. Later, the mice were allowed to 18 h of food and water deprivation, followed by the weight of sucrose; water were masured after 1 h. The weight ratio of sucrose solution to that of total fluid intake was calculated [27]. TST is used to measure stress affecting depression; thus, the mice were individually suspended by their tail from the tip using adhesive scotch tape in a box, and their behavior was recorded over a 6-min period. At first, suspended mice trended to escape and then adopted an immobile posture; the immobile time was measured. The weight gain of each mouse was measured weekly. In the end, the mice were euthanized by cervical dislocation on the morning of metestrus, and their ovaries were analyzed for primordial, primary, secondary, and antral follicles.

2.4. Histological analysis

Ovaries were fixed in 10 % formaldehyde at 4 °C. Tissues were dehydrated in a graded alcohol series, followed by clearing in xylene and embedding in paraffin wax. Ovaries were cut serially in 7 μm thickness from each ovary along the long axis of the entire ovary. Finally, glass slides were stained with hematoxylin and eosin. Next, the stained sections were evaluated by an expert unaware of the treatments. The follicles were categorized as primordial, primary, secondary, and antral follicles. Primordial follicles contained an oocyte surrounded by a single layer of flattened follicular cells. When granulosa cells became squamous or cuboidal shapes, follicles were classified as primary. Oocyte surrounded by 2 or 3 layers of granulosa cells were classified as secondary. Follicles with a markedly enlarged antrum and a visible follicular antrum were classified as antral follicles. The number and diameter of each follicle (primordial, primary, secondary, and Antral) were evaluated and finally compared among groups [28].

2.5. Statistical analysis

Data analysis was performed using the statistical software SPSS 20.0. Differences between body weight, behavioral tests, as well as the number and diameter of follicles, in the control and experimental groups were compared using a two-sample Student t-test or one-way analysis of variance (ANOVA), followed by a Tukey post hoc test. A P < 0.05 was considered statistically significant, and data were presented as mean ± SEM. All graphs were performed with GraphPad Prism 8.0 software.

3. Results

As observed in Fig. 1A, the mean of the body weight of the control and treated groups was significantly different (p ≤0.05). The results showed that body weight gain in the stress groups was persistently lower than the control group after being exposed to stressors for 4 weeks. This was first evident by the end of week 3.

According to Fig. 1B, after 4 weeks of stimulation, the duration of immobility in FST depressive-like behaviors in stress-induced groups was increased in comparison with the control group (p = 0.034). The percentage of sucrose preference in control mice showed greater data deviation compared to the I, II, and III groups (p = 0.036) (Figure 1C). The immobility results in TST showed mice in the I, II, and III groups to have significantly lower mobility than those in the control group (p = 0.016) (Fig. 1D).

Histopathological results are shown in Fig. 2. According to the obtained results of the stress-induced model, the follicle number was different in experimental groups compared to the control. Primordial follicle number showed a significant decrease in the I and III groups compared to the control (p = 0.034). The I and III groups displayed fewer primary numbers compared to mice in the control group after 4 weeks (p = 0.02). Also, there was a significant decrease in the number of secondary follicles in the I and III mice (p <0.001). In the I and III groups, the number of antral follicles was significantly decreased compared to the control (p =0.041). Furthermore, no significant difference was observed in the number of primordial, primary, secondary, and antral follicles in the II group after stress-induced depression (Fig. 2A). In addition, there was a remarkable decreasing trend in the diameter of secondary and antral follicles after 4 weeks in the I and III groups (p = 0.039); however, the diameter of primordial and primary follicles was not varied (Fig. 2B). Also, no significant alteration was observed in the diameter of the primordial, primary,
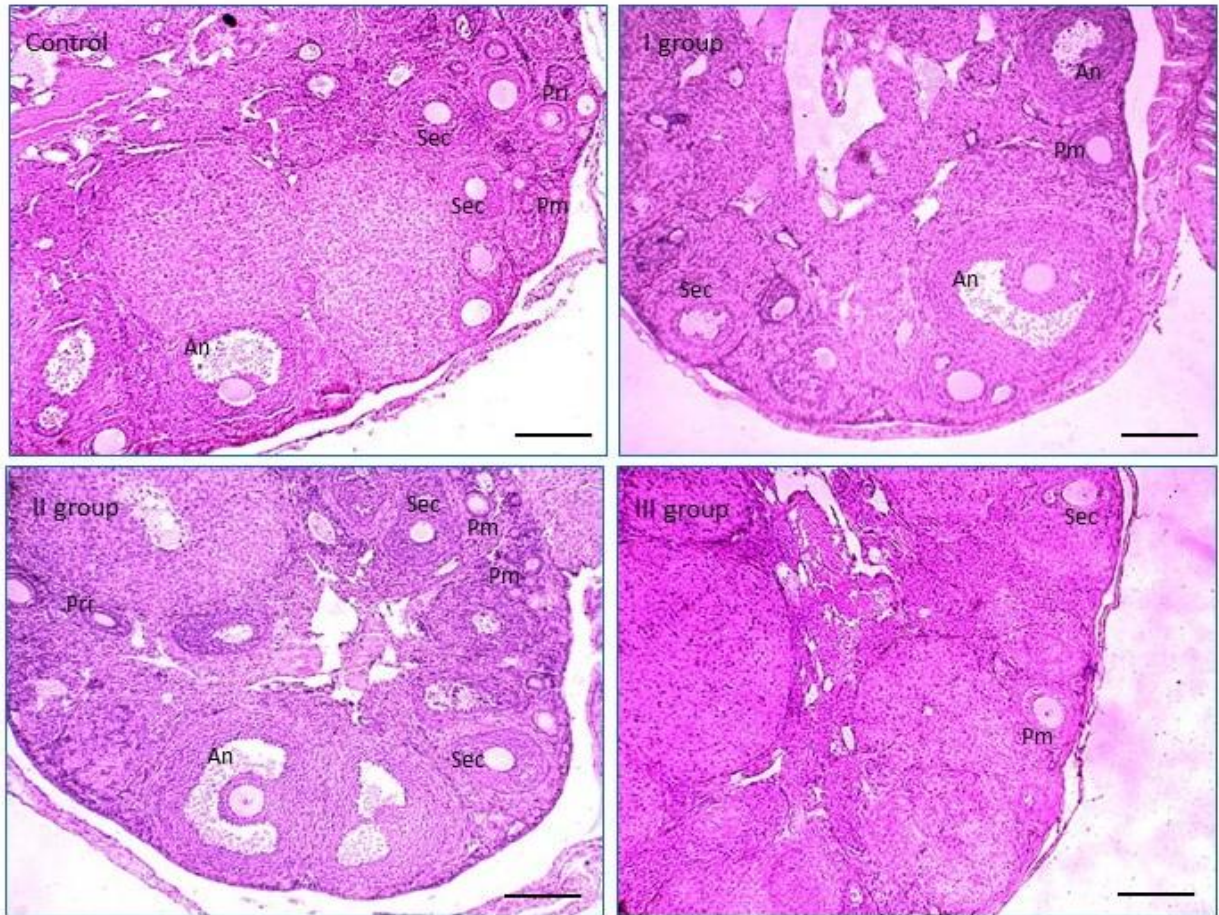
secondary, and antral follicles in the II group compared to the control (Fig. 2B).
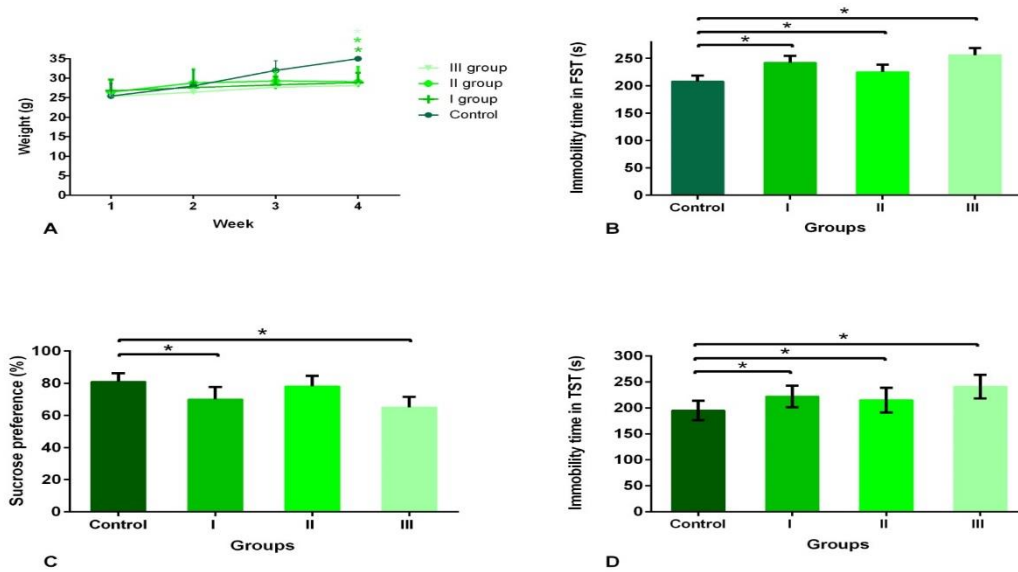
Fig.1 Oral administration of pistachio oil (*Pistacia vera*) to the mice model undergoing chronic unpredictable mild stress: A) Weight changes, B) Immobility time (in seconds) in the forced swim test, C) Percentage of sucrose preference, D) Immobility time in tail suspension test; The mice in experimental groups (I, II, and III groups) were exposed to a variety of chronic unpredictable stress for 4 weeks. The mice in I and II groups were fed orally 1 and 4 ml/kg/day pistachio oil for 4 weeks, respectively. Animals in the III group received tap water. Animals in the control group received water intragastrically (n=8 animals in each group). *p* values < 0.05 as compared to control animals.
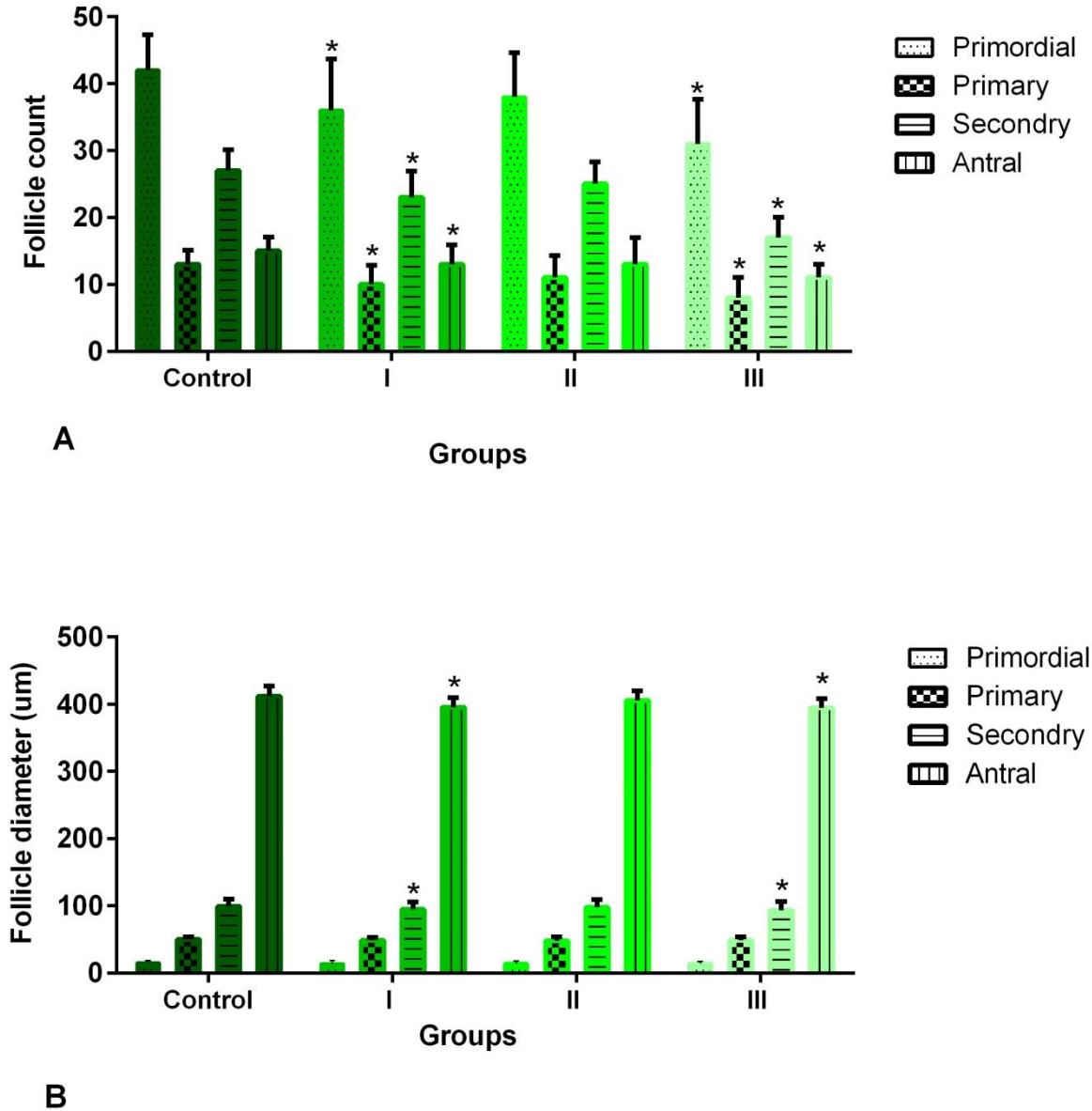
Fig. 2 Oral administration of pistachio oil (*Pistacia vera*) to the mice model undergoing chronic unpredictable mild stress: A) The number of primordial, primary, secondary, and antral follicles, B) The diameter of primordial, primary, secondary, and antral follicles; The mice in experimental groups (I, II, and III groups) were exposed to a variety of chronic unpredictable stress for 4 weeks. The mice in the I and II groups were fed orally 1 and 4 ml/kg/day pistachio oil for 4 weeks, respectively. Animals in the III group received tap water. Animals in the control group received water intragastrically (n=8 animals in each group). *p* values < 0.05 as compared to control animals.

Fig. 3 Histopathological results after oral administration of pistachio oil (*Pistacia vera*) to the mice model undergoing chronic unpredictable mild stress (:A) The control group received water intragastrically, B) the I group was fed orally 1 ml/kg/day pistachio in a variety of chronic unpredictable stress for 4 weeks, C) The II group was fed orally 4 ml/kg/day pistachio oil in a variety of chronic unpredictable stress for 4 weeks, D) The III group was exposed to a variety of chronic unpredictable stress for 4 weeks and received tap water. An: antral follicle, Pri: primary follicle, Pm: primordial follicle, Sec: secondary follicle; Scale bar = 100um.

3. Discussion

In this study, we evaluated the effects of pistachio oil (*Pistacia vera*) on follicle development in 57BL/6J mice models undergoing chronic unpredictable mild stress. The results of body weight showed that weight significantly decreased in stressed animals compared to the control group. Several studies have indicated that stress has a negative effect on body weight [29, 30]. Further, some studies have discussed that stress negatively affects food intake, which is the main factor for weight reduction. However, to date, the mechanism of body weight changes in stress remains unidentified [31, 32].

Stress is one of the most common problems related to reduced life happiness and decreased functional reaction [33]. The present study showed the pistachio oil to have a positive effect on immobility in FST in mice stress model. There was a significant difference between FST in stress-induced groups and the control. Also, there was no significant difference between the experimental group that received 4 mg/kg pistachio oil and the control group in FST. Previous reports have demonstrated that immobility in FST is higher in stress model animals than those normal [34, 35]; this is in line with our findings. Hakimizadeh et al. (2019) have evaluated the effect of pistachio on behaviors in the mice menopause model. They report that 10 and 100 mg/kg pistachio could reduce anxiety-like behavior in menopause model animals [36].

In a study, researchers demonstrate the effect of pistachio on anxiety in Wistar rats. Their results show that tannin and flavonoids in pistachio hydroalcoholic extract can decrease the level of anxiety in animals [37]. The results of another study (2014) indicate that pistachio can play an essential role in depression control; according to the research results, the level of omega 3 in pistachio is possibly the effective factor in depression control [38].

The results of the present study showed TST in stress model groups to be significantly higher than the control group. Pistachio oil (4 mg/kg) could significantly decrease TST compared to other groups under chronic stress.

Studies have shown that stress can increase TST in experimental animals. Rabiei et al. (2017) evaluate the effect of grapeseed oil on behavioral factors in depressed mice. Their results show TST to be lower in the control group than the stress model mice; this is consistent with our findings. They indicate that grapeseed oil (60 mg/kg) decreases TST in depressed mice; they further conclude that depression and stress increase the level of inflammatory factors, including interleukin-6 and tumor necrosis alpha (TNFα). Several fatty acids in grapeseed can decrease these inflammatory factors in stress model mice [39]. Some of its fatty acids and compounds are similar to pistachio, being possibly responsible for the similarity in results.

Histologic evaluation in the present study showed that primordial, primary, secondary, and antral follicles in stressed animals decreased, and when animals were treated with pistachio oil (4 mg/kg), the number of follicular cells increased.

In the human body, elevation in reactive oxygen species (ROS) is related to decreased antioxidant defense, thus affecting physiological functions negatively [40]. It has been established that stress and depression can increase the level of ROS in the body. ROS affects all tissues and systems, including the brain, heart, endocrine, and reproductive system [41].

Previous research has shown that compounds in pistachio oil inhibit the production of nitric oxide (NO). NO is the main factor that can decrease the secretion of

hormones and prevent the steroidogenesis pathway [20].

Recently, Behmanesh et al. (2021) have shown that P. atlantica pistachio can be used to treat streptozotocin-induced diabetic rats in order to reduce ovarian complications. They conclude that the oxidative stress (OS) pathways are activated in diabetic cases, affecting ovarian function negatively. Also, they state that P. atlantica pistachio possibly increases antioxidant activity, which reduces OS levels. Therefore, the reduction of OS is effective in ovaries function [42].

In another study, Mohammadi-Nasab et al. evaluate the effects of pistachio oil on depression and similar anxiety behaviors in female rats with letrozole-induced PCOS. The FST test shows that PCOS conditions lead to increased immobilization time in the FST. PCOS animals show significant anxiety behaviors. Pistachio oil of 1 and 4 ml/kg completely decreases behavioral parameters; it can decrease depression and anxiety in mice[43].

Shariati et al. (2013) evaluate the effects of pistachio oil on the pituitary-gonadal axis and testicular tissue changes in adult male rats. Wistar rats are given pistachio oil in doses of 1, 2, and 4 ml/kg orally for 28 days. Their results show that 2 and 4 ml/kg pistachio oil significantly increase testosterone levels compared to the control group (p <0.05). Testicular histological examinations show that sperm cells are increased in seminiferous tubules of experimental groups compared with the control group (p <0.05) [44]. Although they evaluate male rats and we do female mice, the results are similar.

4. Conclusion

The present study shows that pistachios have positive effects on depression and stress behaviors in mice. Also, pistachio oil can protect follicular cells from the harmful effects of stress.

Conflict of interest

The authors of this article have no conflict of interest.

Acknowledgments

References

1- Ceran MU, Yilmaz N, Ugurlu EN, Erkal N, Ozgu-Erdinc AS, Tasci Y, et al. Psychological domain of quality of life, depression and anxiety levels in in vitro fertilization/intracytoplasmic sperm injection cycles of women with endometriosis: a prospective study. J Psychosom Obstet Gynaecol. **2020**;*8:1-8.*

2- Akaiwa M, Fukui E, Matsumoto H. Tubulointerstitial nephritis antigen-like 1 deficiency alleviates age-dependent depressed ovulation associated with ovarian collagen deposition in mice. Reprod Med Biol. **2019**;*19(1):50-7.*

3- Slavich GM, Sacher J. Stress, sex hormones, inflammation, and major depressive disorder: Extending Social Signal Transduction Theory of Depression to account for sex differences in mood disorders. Psychopharmacology. **2019**;*236(10):3063-79.*

4- Merz CJ. Contribution of stress and sex hormones to memory encoding. Psychoneuroendocrinology. **2017**;*82:51-8.*

5- Merz CJ, Wolf OT. Sex differences in stress effects on emotional learning. Journal of Neuroscience Research. **2017**;*95(1-2):93-105.*

6- Palanza P, Parmigiani S. How does sex matter? Behavior, stress and animal models of neurobehavioral disorders. Neuroscience & Biobehavioral Reviews. 2017;76:134-43.

7- Valsamakis G, Chrousos G, Mastorakos G. Stress, female reproduction and pregnancy. Psychoneuroendocrinology. **2019**;*100:48-57.*

8- Di Natale MR, Soch A, Ziko I, De Luca SN, Spencer SJ, Sominsky L. Chronic predator stress in female mice reduces primordial follicle numbers: implications for the role of ghrelin. Journal of Endocrinology. **2019**;*241(3):201-19.*

9- Culig L, Surget A, Bourdey M, Khemissi W, Le Guisquet A-M, Vogel E, et al. Increasing adult hippocampal neurogenesis in mice after exposure to unpredictable chronic mild stress may counteract some of the effects of stress. Neuropharmacology. **2017**;*126:179-89.*

10- Zhao X, Cao F, Liu Q, Li X, Xu G, Liu G, et al. Behavioral, inflammatory and neurochemical disturbances in LPS and UCMS-induced mouse models of depression. Behavioural brain research. **2019**;*364:494-502.*

11- Brooks SD, Hileman SM, Chantler PD, Milde SA, Lemaster KA, Frisbee SJ, et al. Protection from chronic stress-and depressive symptom-induced vascular endothelial dysfunction in female rats is abolished by preexisting metabolic disease. American Journal of Physiology-Heart and Circulatory Physiology. **2018**;*314(5):H1085-H97.*

12- Xu HX, Lin SX, Gong Y, Huo ZX, Zhao CY, Zhu HM, et al. Chaiyu-Dixian Formula Exerts Protective Effects on Ovarian Follicular Abnormal Development in Chronic Unpredictable Mild Stress (CUMS) Rat Model. Front Pharmacol. **2020**;*11(245):1-18.*

13- Fernandois D, Na E, Cuevas F, Cruz G, Lara H, Paredes A. Kisspeptin is involved in ovarian follicular development during aging in rats. The Journal of endocrinology. **2015**;*228(3):161-70.*

14- Begni V, Riva MA, Cattaneo A. Cellular and molecular mechanisms of the brain-derived neurotrophic factor in physiological and pathological conditions. Clinical Science. **2017**;*131(2):123-38.*

15- de Almeida AA, Gomes da Silva S, Lopim GM, Vannucci Campos D, Fernandes J, Cabral FR, et al. Physical exercise alters the activation of downstream proteins related to BDNF-TrkB signaling in male Wistar rats with epilepsy. Journal of Neuroscience Research. **2018**;*96(5):911-20.*

16- Liu Y, Qin X, Lu X, Jiang J. Effects of inhibiting the PI3K/Akt/mTOR signaling pathway on the pain of sciatic endometriosis in a rat model. Canadian journal of physiology and pharmacology. **2019**;*97(10):963-70.*

17- Hakimizadeh E, Kaeidi A, Hassanshahi J, Mehrbani M, Rahmani M. Ameliorating effect of pistachio hydroalcoholic extract on cisplatin-induced nephrotoxicity in mice.

Research Journal of Pharmacognosy. **2021**;*8(1):73-9*.

18- Abbasifard M, Bagheri-Hosseinabadi Z. Anti-Inflammatory Activities of Pistachio. Pistachio and Health Journal. **2020**;*3(4):50-62*.

19- Hazrati S, Govahi M, Ebadi M-T, Habibzadeh F. Comparison and evaluation of oil content, composition and antioxidant properties of Pistacia atlantica and Pistacia khinjuk grown as wild. International Journal of Horticultural Science and Technology. **2020**;*7(2):165-74*.

20- Oskoueian E, Karimi E, Noura R, Ebrahimi M, Shafaei N, Karimi E. Nanoliposomes encapsulation of enriched phenolic fraction from pistachio hulls and its antioxidant, anti-inflammatory, and anti-melanogenic activities. Journal of microencapsulation. **2020**;*37(1):1-13*.

21- Arjeh E, Akhavan H-R, Barzegar M, Carbonell-Barrachina ÁA. Bio-active compounds and functional properties of pistachio hull: A review. Trends in Food Science & Technology. **2020**;*97:55-64*.

22- Santos HO, Cabral CE, Klein MRST. The need for sex hormone analysis in addition to long-term follow-up of phytosterol supplementation. Arquivos brasileiros de cardiologia. **2018**;*111(2):228-9*.

23- Massimo L, Ginevra L-B. Phytosterols and phytosterol oxides in Bronte's Pistachio (Pistacia vera L.) and in processed pistachio products. European Food Research and Technology. **2020**;*246(2):307-14*.

24- Jamshidi S, Hejazi N, Golmakani M-T, Tanideh N, Esfahani MH. Preventive Effect of Different Wild Pistachio Oils on Oxidative Stress Markers, Liver Enzymes, and Histopathological Findings in a Metabolic Syndrome Model. Galen Medical Journal. **2019**;*8:1238*.

25- Zhang Y, Wang Y, Lei H, Wang L, Xue L, Wang X, et al. Optimized animal model to mimic the reality of stress-induced depression in the clinic. BMC psychiatry. **2017**;*17(1):1-9*.

26- Au - Can A, Au - Dao DT, Au - Arad M, Au - Terrillion CE, Au - Piantadosi SC, Au - Gould TD. The Mouse Forced Swim Test. JoVE. **2012** ;*29(59):e3638*.

27- He LW, Zeng L, Tian N, Li Y, He T, Tan DM, et al. Optimization of food deprivation and sucrose preference test in SD rat model undergoing chronic unpredictable mild stress. Animal models and experimental medicine. **2020**;*3(1):69-78*.

28- Barzegar MH, Khazali H, Kalantar SM, Khoradmehr A. Effect of Citrullus colocynthis hydroalcoholic extract on hormonal and folliculogenesis process in estradiol valerate-induced PCOs rats model: An experimental study. International Journal of Reproductive BioMedicine. **2017**;*15(10):661*.

29- Son H, Kim S, Lee Y. Suggesting coping strategies for the various stresses from body weight in Korean males-A qualitative approach. Journal of the Korean Society of Clothing and Textiles. **2018**;*42(5):884-96*.

30- Osti R, Bhattarai D, Zhou D. Climatic variation: effects on stress levels, feed intake, and bodyweight of broilers. Brazilian Journal of Poultry Science. **2017**;*19(3):489-96*.

31- Sinha R. Role of addiction and stress neurobiology on food intake and obesity. Biological psychology. **2018**;*131:5-13*.

32- Hill DC, Moss RH, Sykes-Muskett B, Conner M, O'Connor DB. Stress and eating behaviors in children and adolescents: Systematic review and meta-analysis. Appetite. **2018**;*123:14-22*.

33- Singh MM, Amiri M, Sabbarwal S. Role of Job Stress on Job Satisfaction. International Journal of Management Studies. **2019**;*6(4):57-60*.

34- Nakatake Y, Furuie H, Yamada M, Kuniishi H, Ukezono M, Yoshizawa K, et al. The effects of emotional stress are not identical to those of physical stress in mouse model of

social defeat stress. Neuroscience research. **2020**;*158:56-63.*

35- Rincón-Cortés M, Grace AA. Sex-dependent effects of stress on immobility behavior and VTA dopamine neuron activity: modulation by ketamine. International Journal of Neuropsychopharmacology. **2017**;*20(10):823-32.*

36- Hakimizadeh E, Jandaghi F, Hajmohammadi M, Fatemi I, Kaeidi A, Shamsizadeh A. Pistachio extract improves neurocognitive behaviors in ovariectomized mice. Research Journal of Pharmacognosy. **2019**;*6(4):45-51.*

37- Rostampour M, Hadipour E, Oryan S, Soltani B, Saadat F. Anxiolytic-like effect of hydroalcoholic extract of ripe pistachio hulls in adult female Wistar rats and its possible mechanisms. Research in pharmaceutical sciences. **2016**;*11(6):454-60.*

38- de Oliveira DR, Zamberlam CR, Gaiardo RB, Rêgo GM, Cerutti JM, Cavalheiro AJ, et al. Flavones from Erythrina falcata are modulators of fear memory. BMC complementary and alternative medicine. **2014**;*14(1):1-17.*

39- Rabiei Z, Naderi S, Rafieian-Kopaei M. Study of antidepressant effects of grape seed oil in male mice using tail suspension and forced swim tests. ||| Bangladesh Journal of Pharmacology. **2017**;*12(4):397-402.*

40- Iqbal H, Yaning C, Waqas M, Shareef M, Raza ST. Differential response of quinoa genotypes to drought and foliage-applied H2O2 in relation to oxidative damage, osmotic adjustment and antioxidant capacity. Ecotoxicology and environmental safety. **2018**;*164:344-54.*

41- Maes M, Galecki P, Chang YS, Berk M. A review on the oxidative and nitrosative stress (O&NS) pathways in major depression and their possible contribution to the (neuro) degenerative processes in that illness. Progress in Neuro-Psychopharmacology and Biological Psychiatry. **2011**;*35(3):676-92.*

42- Behmanesh MA, Poormoosavi SM. Pistacia atlantica's effect on ovary damage and oxidative stress in streptozotocin-induced diabetic rats. JBRA assisted reproduction. **2021**;*25(1):28-33.*

43- Mohammadi-Nasab M, Fatemi I, Pak-Hashemi M, Abbasi-Fard M, Hassanipour M. The Effects of Pistacia Vera Seed Oil on Anxiety and Depressive-Like Behaviors in Rats with Polycystic Ovary Syndrome. Pistachio and Health Journal. 2019;2(1):1-9.

44- Shariati M, Sepehrara L. Effect of pistacia vera oil on pituitary gonad axis and histological testis changes in adult male rats. Armaghane danesh. **2013**;*18(8):641-9.*