ATATÜRK ÜNİVERSİTESİ YAYINLARI
ATATURK UNIVERSITY PUBLICATIONS

Research Article *Araştırma Makalesi* DOI: 10.17094/vetsci.1529099

Protective Effects of Thymol with Hormonal, Anti-inflammatory and Antioxidant Pathways in Lipopolysaccharide-Induced Ovarian Damage in Rats

Sıçanlarda Lipopolisakkarit ile Uyarılan Ovaryum Hasarında Timol'ün Hormonal, Antiinflamatuvar ve Antioksidan Yollarla Koruyucu Etkileri

İshak GÖKÇEK[1]
Gökhan UYANIK[2]

[1]Hatay Mustafa Kemal University, Faculty of Veterinary Medicine, Department of Physiology, Hatay, Türkiye
[2]Erciyes University, Faculty of Veterinary Medicine, Department of Obstetrics and Gynaecology, Kayseri, Türkiye

Received/Geliş Tarihi: 06.08.2024
Accepted/Kabul Tarihi: 09.12.2024
Publication Date/Yayın Tarihi:29.04.2025

Corresponding author/Sorumlu Yazar:
İshak GÖKÇEK
E-mail: igokcek@mku.edu.tr, ishakgokcek@hotmail.com

Cite this article: Gökçek İ, Uyanık G. Protective effects of thymol with hormonal, anti-inflammatory and antioxidant pathways in lipopolysaccharide-induced ovarian damage in rats. *Vet Sci Pract*. 2025;20(1):1-7.

Atıf: Gökçek İ, Uyanık G. Sıçanlarda lipopolisakkarit ile uyarilan ovaryum hasarinda timol'ün hormonal, antiinflamatuvar ve antioksidan yollarla koruyucu etkileri. *Vet Sci Pract*. 2025;20(1):1-7.

ABSTRACT

This study investigated the protective effects of thymol on lipopolysaccharide induced ovarian damage. Female Wistar albino rats were divided into five groups (n=35, group=7): control, vehicle, LPS, thymol, and LPS+thymol. At the end of the study, estradiol 17 beta (E2), anti-Mullerian hormone (AMH), oxidative stress parameters such as malondialdehyde (MDA), glutathione (GSH), glutathione peroxidase (GSH-Px), catalase, and inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin 6 (IL-6) were analyzed in samples taken from animals. Ovary and body weights were also measured. Lipopolysaccharide treatment caused decreases in E2, AMH, GSH, GSH-Px and catalase levels but increased MDA, TNF-α and IL-6 levels. In the Lps+thymol group, thymol administration caused increases in E2, AMH, GSH, GSH-Px and catalase levels and decreased MDA, TNF-α and IL-6 levels. In conclusion, thymol administration positively affected Lps-induced hormonal, oxidative stress and inflammatory changes via antioxidant and anti-inflammatory mechanisms. However, it is thought that the long-term effects of thymol need to be demonstrated, especially by further molecular mechanisms. Within this framework, examining the dose-dependent effects of thymol, the outcomes of its application over varying durations, and investigating the biochemical changes at the cellular level will offer valuable insights for future research and elucidate the potential effects of this compound. These findings reveal the therapeutic potential of thymol; nevertheless, further studies are essential for its clinical application.

Keywords: Inflammation, LPS, oxidant-antioxidant status, ovarian reserve, thymol

ÖZ

Bu çalışmada LPS ile uyarılan ovaryum hasarında timolün koruyucu etkileri araştırıldı. Bu amaçla Wistar albino dişi sıçanlar 5 gruba ayrıldı (n=35, grup=7) : kontrol, taşıyıcı, lps, timol, lps+timol. Çalışma sonunda hayvanlardan alınan numunelerde hormon (E2 ve AMH), oksidatif stres (MDA, GSH, GSH-px ve katalaz), yangısal sitokin (TNF-α, IL-6) analizleri gerçekleştirildi. Ayrıca ovaryum ve canlı ağırlıklarda ölçüldü. Lipopolisakkarit uygulaması, E2 AMH, GSH, GSH-px ve katalaz seviyelerinde azalmalara neden olurken, MDA, TNF-α ve IL-6 düzeylerinde artışa neden oldu. LPS+timol grubunda ise timol uygulaması E2 AMH, GSH, GSH-px ve katalazda artışlara, MDA, TNF-α ve IL-6 seviyelerinde ise azalmalara neden olduğu görüldü. Sonuç olarak timol uygulaması, antioksidan ve anti-inflamatuvar mekanizmalar üzerinden Lps kaynaklı hormonal, oksidatif stres, yangısal değişiklikleri olumlu olarak etkilediği ancak timolün uzun süreli etkilerinin özellikle daha ileri moleküler mekanizmalarla ortaya konmasına ihtiyaç olduğu düşünülmektedir. Bu bağlamda, timolün doz-bağımlı etkilerinin araştırılması, farklı sürelerde uygulanmasının sonuçları ve hücresel düzeydeki biyokimyasal değişimlerin incelenmesi, gelecekteki çalışmalara ışık tutacak ve bu bileşiğin potansiyel etkilerini ortaya koyacaktır. Bu bulgular, timolün terapötik potansiyelini ortaya koymaktadır, ancak klinik uygulamalar için daha fazla araştırma gereklidir.

Anahtar Kelimeler: LPS, oksidan-antioksidan durum, ovarium rezerv, timol, yangı

INTRODUCTION

Microbial environment, metabolites and immune system components coordinate in reproductive system homeostasis.[1] In the postpartum period, the female reproductive tract is highly vulnerable to bacteria, which can easily invade the uterine flora and cause various reproductive pathologies like metritis and endometritis.[2,3] Lipopolysaccharide (LPS), one of the main components of Gram-negative bacteria's outer membrane, can harm reproductive functions such as follicular and embryonic development, ovulation and implantation.[4-7] Also, oxidative stress, inflammation and apoptosis pathways negatively affect ovarian functions like steroidogenesis, folliculogenesis, ovulation, and luteolysis.[8]

Oxidative stress is reported to be involved in the pathogenesis of various female reproductive disorders.[9,10] Lipopolysaccharides cause reactive oxygen species (ROS) accumulation and lipid peroxidation and may damage antioxidant defense systems.[11] It is also known that oxidative stress is associated with inflammation and induces inflammation.[12] The increase in ROS can also stimulate the secretion of tumour necrosis factor-alpha (TNF-α) in oocytes and granulosa cells.[13] In addition, LPS causes immune responses through different molecular pathways.[14] It is known that lipopolysaccharide-induced inflammatory condition causes damage to ovaries and interferes with female reproductive activities.[8] For example, it is stated that inflammatory conditions negatively affect ovarian follicle dynamics.[12]

Thymol, one of the main components of thyme, frequently used in the Mediterranean Diet (MD), is a monoterpene phenolic compound with antioxidant, anti-inflammatory, local anaesthetic, antinociceptive, antiseptic, antibacterial, and antifungal properties.[15] Thymol positively affects the reproductive system in both females and males.[16,17] In a study conducted, thymol caused improvement in sperm parameters in male rats.[16] A study observed high embryo rates in in vitro fertilization in women adhering to the MD.[18] A monkey study showed that the MD caused regular menstrual cycles compared to the Western diet.[19] In addition, the beneficial effects of thymol have been reported in female reproductive disorders such as polycystic ovary syndrome.[20] A study also reported that thymol suppressed the inflammatory response in radiation-induced ovarian damage in rats.[17]

This study aimed to investigate the protective effects of thymol against LPS-induced ovarian damage. For this objective, hormonal, inflammatory, oxidant, and antioxidant status were analyzed, and the protective effects of thymol on female reproductive activities were aimed to be revealed.

MATERIALS AND METHODS

Experimental Design

Female Wistar albino rats (200-250 g) were used in the study (n=35, per 7): control (C), vehicle (V), lipopolysaccharide (LPS), thymol (TML), lipopolysaccharide+thymol (LPS+TML). The experimental model lasted seven days. The TML and LPS+TML groups were given thymol (20 mg/kg/p.o) daily for seven days starting from the first day of the study.[16] Also, the LPS and LPS+TML groups received LPS (100 µg/kg/i.p) four times every second day, starting on the first day of the study, by modifying the model specified by Pal et al.[8] The Vehicle group was given 5% dimethyl sulfoxide (DMSO), used as a thymol solvent, daily throughout the study. (Ethics Committee Decision No: 2023/09-3, Date: 21.12.2023)

At the end of the study, blood was taken from the animals' heart chambers by the intracardiac method under xylazine ketamine anaesthesia. Then, ovarian samples were collected after euthanasia. The obtained blood measured the E_2 and AMH levels. The ovarian tissues measured the levels of TNF-α, IL-6, MDA, GSH, GSH-Px, and catalase. Also, the animals' body and ovarian weights were weighed at the end of the study.

Ovarian and Body Weight Weighing

Right and left ovary samples were thoroughly cleaned, weighed, averaged and expressed in milligrams. Body weights were also weighed and expressed in grams.

Hormone Analyses (E2 and AMH)

Blood samples were centrifuged at 1000 x g for 20 minutes, and sera were collected. E2 levels in the serum samples were measured by electrochemiluminescence immunological method. Serum AMH levels were also measured with a commercial ELISA kit (Finetest, Cat No: ER0260) and expressed as ng/ml.

Ovarian Oxidative Stress Analyses (MDA, GSH, GSH-Px and Catalase)

Both ovaries were brought together and homogenized with 1/10 (weight/volume) Tris-buffered saline (pH 7.4).[17] The homogenate obtained was centrifuged, and the supernatant was collected. All oxidative stress analyses were performed on these supernatant portions.[16] Protein concentrations in the samples were also measured with a commercial BCA assay kit. The Shimadzu UV1700 spectrometer was used for all oxidative stress analyses.

Malondialdehyde levels were measured at 532 nm

according to the method of Placer et al.[22] GSH levels were measured at 412 nm in the spectrometer according to the method of *Sedlak and Lindsay*.[23] MDA and GSH levels are also expressed as nmol/gr protein. GSH-Px was performed according to *Lawrence and Burk*'s method[24], and samples were measured at 340 nm and expressed as IU/gr protein. Catalase was performed according to the method described by Aebi.[25] and expressed as kU/gr protein.

Inflammatory Cytokine Analyses (TNF- α and IL-6)

The ovarian samples (right and left) were homogenized after diluting 1/10 (weight/volume) with phosphate buffered saline (pH 7.4).[17] The homogenate obtained was centrifuged at 5000 g for 5 min at +4°C, and the supernatant was collected. TNF-α (Finetest, Cat No: ER1393) and IL-6 (Finetest, Cat No: ER0042) were measured in this supernatant according to the method specified by ELISA kits. In addition, total protein levels of ovarian tissue were measured by a commercial BCA protein kit. TNF-α and IL-6 levels were expressed as pg/mg protein.

Statistical Analysis

Parametric test assumptions were applied for all variables obtained before proceeding to significance tests. The variables were analyzed using the Shapiro-Wilk test for normality and Levene's test for homogeneity. Then, one-way analysis of variance (ANOVA) was used to control the statistical difference between variables. The Duncan test was used as a post hoc test for the variables in which the difference between the groups was significant. All statistical analyses were performed with a minimum 5% margin of error. IBM SPSS 23 (IBM SPSS Corp., Armonk, NY, USA) was used in all statistical analyses.

RESULTS

Ovarian and Body Weight

The ovarian weights of the C, V, LPS, TML, and LPS+TML groups were measured as 72.514±1.321, 71.690±1.424, 70.043±1.117, 72.062±1.182, and 71.378±1.162, respectively. There was no statistically significant difference between the groups (p=0.690). The mean ovarian weights (Mean±SEM) are shown in Figure 1A.

Body weights of C, V, LPS, TML and LPS+TML groups were 239.714±2.679, 241.429±3.023, 223,571±2.819, 241.714±4.034 and 230.286±2.758 grams, respectively. The lowest body weight averages were in the LPS and LPS+TML groups. Also, the other groups (V and TML) were like the control (P < .001). Body weight averages (Mean±SEM) are shown in Figure 1B.

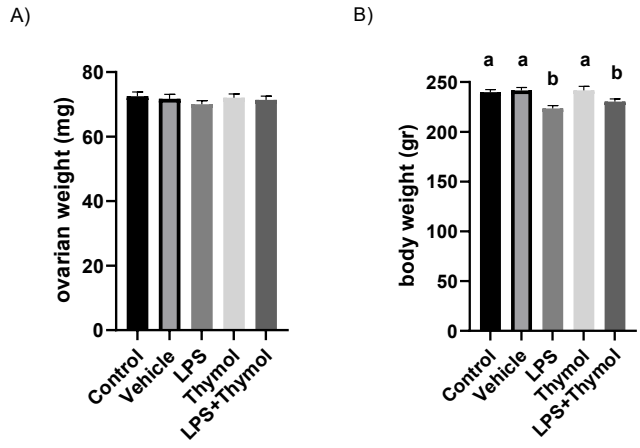

Figure 1. A. Ovary weight averages, **B.** Body weight averages. Letters in the columns indicate statistical differences and similar letters indicate statistical similarity.

Hormone Analyses (E2 and AMH)

E2 hormone levels were 42.351±1.251 in the C group, 42.369±1.535 in the V group, 23.874±1.132 in the LPS group, 43.874±1.901 in the TML group and 33.840±2.327 pg/ml in LPS+TML group. The lowest E2 level among all groups was in the LPS group. In addition, V and TML groups were like the control group, while LPS+TML differed from the control group (P < .001). E2 levels (Mean±SEM) of all groups are shown in Figure 2A.

AMH levels were 1.242±0.041 in the C group, 1.252±0.050 in the V group, 1.058±0.040 in the LPS group, 1.250±0.044 in the TML group, and 1.149±0.047 pg/ml in the LPS+TML group. The LPS group AMH levels were statistically lower than the control group. However, the other groups (V, TML, and LPS+TML) were like the control group (P = .015). The AMH levels (Mean±SEM) of all groups are shown in Figure 2B.

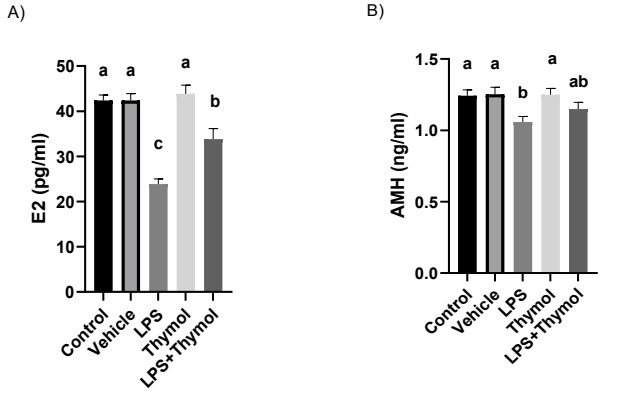

Figure 2. A. Serum E2 levels, **B.** Serum AMH levels. Letters on the columns indicate statistical differences. Similar letters are statistically identical to each other.

Ovarian Oxidative Stress Analyses (MDA, GSH, GSH-Px and Catalase)

Ovarian MDA levels were measured as 2.260±0.045, 2.334±0.100, 3.521±0.057, 2.044±0.073 and 2.836±0.147 nmol/gr protein in all groups, including C, V, LPS, TML, and LPS+TML, respectively. The highest MDA level among all groups was in the LPS group. While V and TML groups were like the control group, the LPS+TML group differed from the control group (P = .001). MDA levels (Mean±SEM) of ovarian tissue are shown in Figure 3A.

GSH levels of the C, V, LPS, TML, and LPS+TML groups were measured as 10.000±0.368, 9.738±0.281, 7.051±0.234, 11.052±0.244, and 8.047±0.153 nmol/gr protein, respectively. The highest and lowest GSH levels were in the TML and LPS groups. While the V group was like the control, the LPS+TML group differed from the control (P < .001). GSH levels (Mean±SEM) of ovarian are shown in Figure 3B.

GSH-Px levels of C, V, LPS, TML and LPS+TML groups were measured as 56.159±2.063, 55.624±1.794, 40.681±1.134, 57.706±1.496 and 47.338±1.538 IU/gr protein, respectively. The lowest GSH-Px levels were in the LPS group. The V and TML groups were like the control, and the LPS+TML group differed from the control (P < .001). GSH-Px levels (Mean±SEM) of ovarian tissue are shown in Figure 3C.

The catalase levels of the C, V, LPS, TML, and LPS+TML groups were 48.336±1.652, 47.069±1.316, 41.359±0.969, 49.748±1.249, and 43.623±1.732 ku/gr protein, respectively. The lowest levels were in the LPS group. While the V and TML groups were like the control, the LPS+TML group was different from the control (P = .001). The catalase levels of ovarian (Mean±SEM) are shown in Figure 3D.

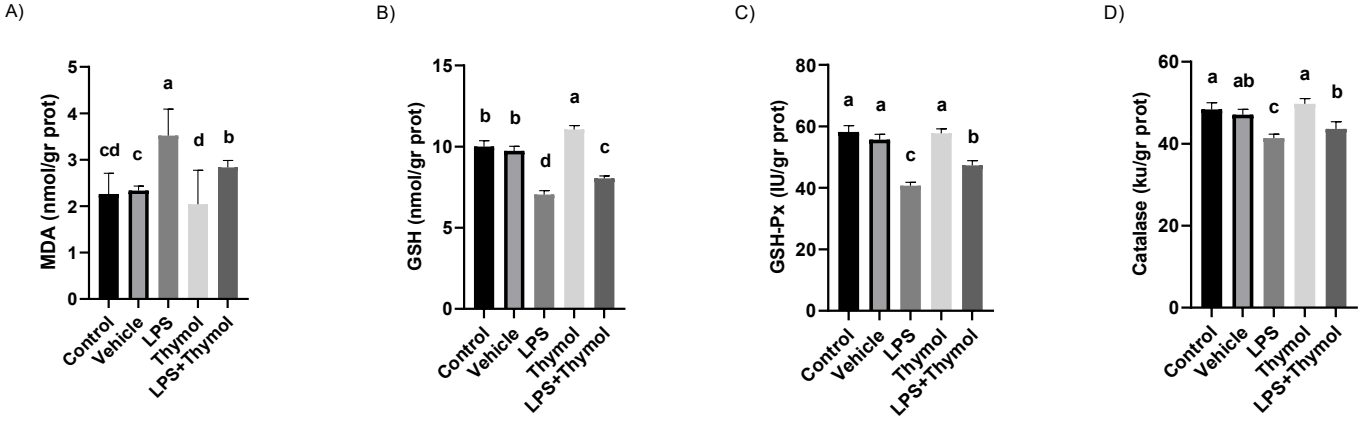

Figure 3. A. Ovary MDA levels, **B.** Ovary GSH levels, **C.** Ovary GSH-Px levels, **D.** Ovary catalase levels. Letters on the columns indicate statistical differences. Similar letters are statistically identical to each other.

Inflammatory Cytokine Analyses (TNF- α and IL-6)

The ovarian TNF-α levels of all groups (C, V, LPS, TML, LPS+TML) were 22.163±0.563, 22.343±0.372, 29.117±1.341, 23.060±0.223 and 25.087±0.733 pg/mg protein, respectively. The highest TNF-α levels were in the LPS group. In addition, while V and TML groups were like the control group, the LPS+TML group differed from the control group (P < .001). TNF-α levels (Mean±SEM) of ovarian tissue are shown in Figure 4A.

The ovarian IL-6 levels of all groups (C, V, LPS, TML, LPS+TML) were measured as 10.001±0.346, 11.104±0.300, 18.813±0.534, 9.187±0.362 and 14.501±0.243 pg/mg protein, respectively. The highest IL-6 levels were in the LPS group. While the TML group was like the control group, V and LPS+TML groups differed from the control group (P < .001). IL-6 levels of ovarian tissue (Mean±SEM) are shown in Figure 4B.

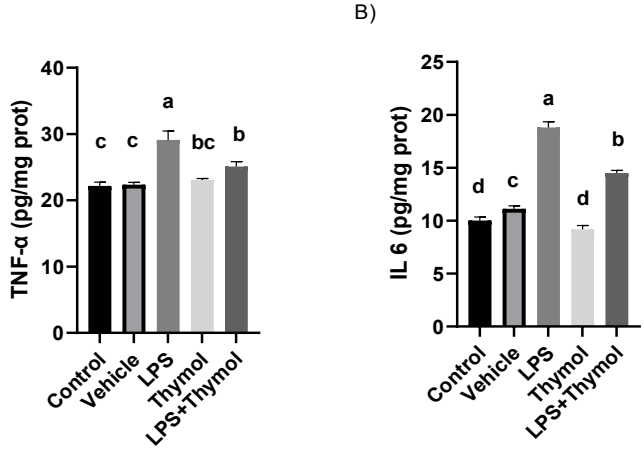

Figure 4. A. Ovary TNF-α levels, **B.** Ovary IL-6 levels. The letters in the columns indicate statistical differences. Similar letters are statistically identical.

DISCUSSION

Although inflammatory processes can lead to follicle depletion, decreased oocyte quality and infertility, controlling this condition positively affects reproductive health.[26-28] Proinflammatory cytokines and reactive oxygen species affect the estrous cycle, steroidogenesis, ovulation, damage oocyte maturation, and embryo development.[29] Previous studies show that LPS cause hormonal imbalances, ovarian failure and infertility in mammals.[30] Similarly, in this study, it is observed that LPS-induced inflammatory condition causes decreases in reproductive hormone levels and disruptions in biochemical parameters in the ovary.

Both low and high doses of LPS prolong the estrous cycle, reduce the number of primordial follicles and change reproductive hormone levels.[31] In a study, it was conveyed that LPS administration caused decreases in serum estrogen and progesterone levels.[8] In another study, it was reported that LPS administration caused decreases in serum estrogen levels.[30] In addition, decreases in LH release occur in inflammation caused by intrauterine LPS.[32] A study stated that LPS administration causes decreases in AMH and E_2 levels in mice.[31] A study even reported that LPS-induced maternal inflammation caused decreases in AMH levels in female offspring mice.[33] In this study, similar to previous studies, LPS administration caused statistically significant decreases in AMH and estrogen levels. Also, it was observed that thymol treatment statistically increased estrogen levels and caused increases in AMH levels.

In a normal estrus, cytokines and chemokines released under physiological conditions are necessary for follicular growth and ovulation.[12,29] Inflammatory mediators are produced during folliculogenesis and participate in ovulation processes.[12] For instance, it is reported that physiological inflammation shaped by the increase in gonadotropin causes weakening and rupture in the follicle wall and contributes to ovulation.[34] C-reactive protein (CRP), another immune system component, is associated with follicular dynamics; women with three follicular waves have higher CRP levels.[35] In addition, bradykinin levels increase approximately 10-fold in ovulation.[34] However, pathological cytokine and chemokine signaling could cause anovulation and infertility by affecting follicular dynamics and impairing oocyte quality.[12,29] Both low and high doses of LPS increase serum and ovarian inflammatory cytokine levels in mice.[31] A study reported decreased follicles with ageing were associated with inflammation in mice.[28] Also, chronic inflammation has harmful effects on follicular dynamics and ovulation.[12] Increasing follicular content inflammatory mediator levels in women receiving infertility treatment causes a decrease in oocytes and a decrease in implantation success.[36] Similar to previous studies, this study found that LPS administration caused statistically significant increases in ovarian TNF-α and IL-6 levels.

In normal physiological conditions, free radicals and antioxidants are in balance, and in oxidative stress situations that occur in the oxidant direction of this balance, oocyte ageing decreases in oocyte quality and quantity, and impairments in embryonic development may occur.[37] Increases in the oxidant level of follicular fluid in women reduce the success of assisted reproductive techniques (ART).[38,39] Also, the granulosa cell antioxidant content of women receiving invitro fertilisation treatment is low.[40] A study observed that LPS administration caused decreases in ovarian superoxide dismutase and catalase levels and increased MDA levels in hamsters.[8] Like other studies, LPS administration caused a statistically significant increase in MDA levels and decreased antioxidant enzyme levels of GSH, GSH-Px and catalase in ovaries.

Antioxidant therapies are among the different protocols for the protection of oocytes.[37] The main principle of treatment strategies involving antioxidants such as antioxidant monomers or melatonin is to reduce oxidative stress and improve ovarian function.[41] Natural antioxidants cause protective effects on ovaries through multiple mechanisms.[42] Thymol, one of the natural antioxidants, can reduce oxidant capacity.[43] For example, a study conducted in rats reported that thymol increased total antioxidant capacity by 115% in radiation-induced oxidative stress.[17] Another study reported that Thymus vulgaris with thymol content showed an antioxidant effect in polycystic ovary syndrome in rats.[44] In line with the previous study, thymol administration in LPS-induced ovarian damage statistically decreased MDA levels, increased antioxidant enzyme levels (GSH-GSH-Px and catalase) and stimulated antioxidant mechanisms.

Identical to antioxidant therapy for oxidant status, controlling inflammatory processes is very important for ovarian activities.[32,36,37] Treatment approaches for controlling follicular fluid cytokine content in women are included in the infertility treatment protocol.[36,45] Upon reviewing the literature, it has been observed that thymol induces anti-inflammatory effects through different molecular mechanisms.[46]Another study stated that the inflammatory condition induced by radiation in the ovary returned to normal levels with thymol in rats.[17] Similar to previous studies, this study observed that using thymol in LPS-induced ovarian damage statistically decreased TNF-α and IL-6 levels in ovarian tissue and thus caused the anti-inflammatory effect.

In conclusion, thymol's use in LPS-induced ovarian damage has protective effects through hormonal, antioxidant, and anti-inflammatory mechanisms. However, further molecular analyses should be performed, and the effects of different doses and durations of thymol on ovarian functions should be investigated.

Ethics Committee Approval: Ethics committee approval was received from Hatay Mustafa Kemal University Animal Experiments Local Ethics Committee (Date: 21.12.2023, Approval No: 2023/09-3).

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - İ.G.; Design - İ.G., G.U.; Supervision-İ.G.; Resources - İ.G., G.U.; Data Collection and/or Processing - İ.G., G.U.; Analysis and/or Interpretation - İ.G., G.U.; Literature Search - İ.G.; Writing Manuscript - İ.G.; Critical Review - İ.G., G.U.

Declaration of Interests: The authors declare no conflict of interest.

Funding: The authors declare they have no financial support for this study.

Etik Komite Onayı: Etik izin Hatay Mustafa Kemal Üniversitesi Hayvan Deneyleri Yerel Etik Kurulu tarafından onaylandı (Date: 21.12.2023, Decision No: 2023/09-3).

Hakem Değerlendirmesi: Dış bağımsız.

Yazar Katkıları: Konsept - İ.G; Tasarım - İ.G., G.U.; Denetim - İ.G.; Kaynaklar - İ.G., G.U.; Malzemeler - İ.G., G.U.; Veri Toplama ve/veya İşleme - İ.G., G.U.; Analiz ve/veya Yorum - İ.G., G.U.; Literatür Taraması - İ.G.; Yazma - İ.G.; Eleştirel İnceleme - İ.G., G.U

Çıkar Çatışması: Yazarlar herhangi bir çıkar çatışması olmadığını beyan etmektedir.

Finansal Destek: Yazarlar bu çalışmada herhangi bir finansal destek almadıklarını beyan etmektedir.

REFERENCES

1.Li H, Zang Y, Wang C, et al. The Interaction between microorganisms, metabolites, and immune system in the female genital tract microenvironment. *Front Cell Infect Microbiol.* 2020;10:609488.
2.Turner ML, Healey GD, Sheldon IM. Immunity and inflammation in the uterus. *Reprod Domest Anim.* 2012;47(4):402-409.
3.Rosales E, Ametaj B. Reproductive tract infections in dairy cows: can probiotics curb down the incidence rate? *Dairy.* 2021;2(1):40-64.
4.Deb K, Chaturvedi MM, Jaiswal YK. Gram-negative bacterial LPS induced poor uterine receptivity and implantation failure in mouse: alterations in IL-1beta expression in the preimplantation embryo and uterine horns. *Infect Dis Obstet Gynecol.* 2005;13(3):125-133.
5.Lee AJ, Kandiah N, Karimi K, Clark DA, Ashkar AA. Interleukin-15 is required for maximal lipopolysaccharide-induced abortion. *J Leukoc Biol.* 2013;93(6):905-912.
6.Bromfield JJ, Sheldon IM. Lipopolysaccharide reduces the primordial follicle pool in the bovine ovarian cortex ex vivo and in the murine ovary in vivo. *Biol Reprod.* 2013;88(4):98.
7.Heidari M, Kafi M, Mirzaei A, Asaadi A, Mokhtari A. Effects of follicular fluid of preovulatory follicles of repeat breeder dairy cows with subclinical endometritis on oocyte developmental competence. *Anim Reprod Sci.* 2019;205:62-69.
8.Pal S, Haldar C, Verma R. Melatonin attenuates LPS-induced ovarian toxicity via modulation of SIRT-1, PI3K/pAkt, pErk1/2 and NFκB/COX-2 expressions. *Toxicol Appl Pharmacol.* 2022;451:116173.
9.Valluru L, Dasari S, Wudayagiri R. Role of free radicals and antioxidants in gynecological cancers: current status and future prospects. *Oxid Antioxid Med Sci,* 2014;3(1):15-26.
10.Agarwal A, Aponte-Mellado A, Premkumar BJ, Shaman A, Gupta S. The effects of oxidative stress on female reproduction: a review. *Reprod Biol Endocrinol.* 2012;10:49.
11.Gao H, Yang T, Chen X, Song Y. Changes of Lipopolysaccharide-induced acute kidney and liver injuries in rats based on metabolomics analysis. *J Inflamm Res*. 2021;14:1807-1825.
12.Boots CE, Jungheim ES. Inflammation and human ovarian follicular dynamics. *Semin Reprod Med.* 2015;33(4):270-275.
13.Kong QQ, Wang J, Xiao B, et al. Cumulus cell-released tumor necrosis factor (TNF)-α promotes post-ovulatory aging of mouse oocytes. *Aging.* 2018;10(7):1745-1757.
14.Khan KN, Kitajima M, Hiraki K, et al. Toll-like receptors in innate immunity: role of bacterial endotoxin and toll-like receptor 4 in endometrium and endometriosis. *Gynecol Obstet Invest.* 2009;68(1):40-52.
15.Marchese A, Orhan IE, Daglia M, et al. Antibacterial and antifungal activities of thymol: A brief review of the literature. *Food Chem.* 2016;210:402-414.
16.Güvenç M, Cellat M, Gökçek İ, Yavaş İ, Yurdagül Özsoy Ş. Effects of thymol and carvacrol on sperm quality and oxidant/antioxidant balance in rats. *Arch Physiol Biochem.* 2019;125(5):396-403.
17.Mahran YF, Badr AM, Aldosari A, Bin-Zaid R, Alotaibi HN. Carvacrol and thymol modulate the cross-talk between TNF-α and IGF-1 signaling in radiotherapy-induced ovarian failure. *Oxid Med Cell Longev*. 2019;2019:3173745.
18.Sun H, Lin Y, Lin D, et al. Mediterranean diet improves

embryo yield in IVF: a prospective cohort study. Reprod Biol *Endocrinol.* 2019;17(1):73.
19.Frye BM, Register TC, Appt SE, et al. Differential effects of western versus mediterranean diets and psychosocial stress on ovarian function in female monkeys (Macaca fascicularis). *Psychoneuroendocrinology*. 2023;153:106107.
20.Ghorbani Ranjbary A, Mehrzad J, Talebkhan Garoussi M, Zohdi J. Long term oral administration of oregano essence effectively relieves polycystic ovarian rats through endocrine and inflammatory balance. *Evid Based Complement Alternat Med.* 2022;2022:5303583.
21.Lin GJ, Sytwu HK, Yu JC, et al. Dimethyl sulfoxide inhibits spontaneous diabetes and autoimmune recurrence in non-obese diabetic mice by inducing differentiation of regulatory T cells. *Toxicol Appl Pharmacol.* 2015;282(2):207-214.
22.Placer ZA, Cushman LL, Johnson BC. Estimation of product of lipid peroxidation (malonyl dialdehyde) in biochemical systems. *Anal Biochem.* 1966;16(2):359-364.
23.Sedlak J, Lindsay RH. Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. *Anal Biochem.* 1968;25(1):192-205.
24.Lawrence RA, Burk RF. Glutathione peroxidase activity in selenium-deficient rat liver. *Biochem Biophys Res Commun.* 1976;71(4):952-958.
25.Aebi H. Catalase in vitro. *Methods in Enzymology.* 1984;105:121-126.
26.Imai A, Ichigo S, Matsunami K, Takagi H, Kawabata I. Ovarian function following targeted anti-angiogenic therapy with bevacizumab. *Mol Clin Oncol*. 2017;6(6):807-810.
27.Han SS, Kim YH, Lee SH, et al. Underuse of ovarian transposition in reproductive-aged cancer patients treated by primary or adjuvant pelvic irradiation. *J Obstet Gynaecol Res*. 2011;37(7):825-829.
28.Lliberos C, Liew SH, Zareie P, La Gruta NL, Mansell A, Hutt K. Evaluation of inflammation and follicle depletion during ovarian ageing in mice. *Sci Rep.* 2021;11(1):278.
29.Snider AP, Wood JR. Obesity induces ovarian inflammation and reduces oocyte quality. *Reproduction.* 2019;158(3):79-90.
30.Shen J, Zhao W, Cheng J, et al. Lipopolysaccharide accelerates tryptophan degradation in the ovary and the derivative kynurenine disturbs hormone biosynthesis and reproductive performance. *J Hazard Mater*. 2023;458:131988.
31.Lv SJ, Hou SH, Gan L, Sun J. Establishment and mechanism study of a primary ovarian insufficiency mouse model using lipopolysaccharide. *Anal Cell Pathol.* 2021;2021:1781532.
32.Magata F, Toda L, Sato M, et al. Intrauterine LPS inhibited arcuate Kiss1 expression, LH pulses, and ovarian function in rats. *Reproduction.* 2022;164(5):207-219.
33.Shalom-Paz E, Weill S, Ginzberg Y, et al. IUGR induced by maternal chronic inflammation: long-term effect on offspring's ovaries in rat model-a preliminary report. *J Endocrinol Invest.* 2017;40(10):1125-1131.
34.Espey LL. Current status of the hypothesis that mammalian ovulation is comparable to an inflammatory reaction. *Biol Reprod.* 1994;50(2):233-238.
35.Clancy KB, Baerwald AR, Pierson RA. Systemic inflammation is associated with ovarian follicular dynamics during the human menstrual cycle. *PLoS One.* 2013;8(5):64807.
36.Sarapik A, Velthut A, Haller-Kikkatalo K, et al. Follicular proinflammatory cytokines and chemokines as markers of IVF success. *Clin Dev Immunol.* 2012;2012:606459.
37.Wang L, Tang J, Wang L, et al. Oxidative stress in oocyte aging and female reproduction. *J Cell Physiol.* 2021;236(12):7966-7983.
38.Oyawoye O, Abdel Gadir A, Garner A, Constantinovici N, Perrett C, Hardiman P. Antioxidants and reactive oxygen species in follicular fluid of women undergoing IVF: relationship to outcome. *Hum Reprod.* 2003;18(11):2270-2274.
39.Pekel A, Gönenç A, Turhan NÖ, Kafalı H. Changes of sFas and sFasL, oxidative stress markers in serum and follicular fluid of patients undergoing IVF. *J Assist Reprod Genet*. 2015;32(2):233-241.
40.Tatone C, Carbone MC, Falone S, et al. Age-dependent changes in the expression of superoxide dismutases and catalase are associated with ultrastructural modifications in human granulosa cells. *Mol Hum Reprod.* 2006;12(11):655-660.
41.Yan F, Zhao Q, Li Y, et al. The role of oxidative stress in ovarian aging: a review. *J Ovarian Res.* 2022;15(1):100.
42.Yang L, Chen Y, Liu Y, et al. The role of oxidative stress and natural antioxidants in ovarian aging. *Front Pharmacol.* 2021;11:617843.
43.Beena, Kumar D, Rawat DS. Synthesis and antioxidant activity of thymol and carvacrol based Schiff bases. *Bioorg Med Chem Lett*. 2013;23(3):641-645.
44.Jafarisani M, Masoomikarimi M, Kazemi SS, Mirzaeidelaviz S, Naderi Z, Ahmadi R. Effect of thymus vulgaris ethanol extract, on serum total antioxidant in experimental induced poly cystic ovarian syndrome (PCOs) rats. *Int J Health Stud.* 2016;2(1):30-34.
45.Büscher U, Chen FC, Kentenich H, Schmiady H. Cytokines in the follicular fluid of stimulated and non-stimulated human ovaries; is ovulation a suppressed inflammatory reaction? *Hum Reprod.* 1999;14(1):162-166.
46. Riella KR, Marinho RR, Santos JS, et al. Anti-inflammatory and cicatrizing activities of thymol, a monoterpene of the essential oil from Lippia gracilis, in rodents. *J Ethnopharmacol.* 2012;143(2):656-663.