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The Histopathological Effects of Immobilization Stress on Rat Testis Tissue, and The Protective Role of Lavender Essential Oil

Elfide Gizem BAKIRHAN[1], Ebru ANNAÇ[1], Gülru ESEN[2]

ABSTRACT

Aim: Stress can lead to various physiological and psychological disorders and also has a significant effect on male reproductive health. Although the effects of stress on female fertility have been extensively studied, its impact on male fertility is relatively under-researched. This study set out to examine the potential protective effect of lavender essential oil (LEO) against testicular damage induced by immobilization stress in rats.

Material and Methods: Twenty-four male Wistar Albino rats were randomly divided into four groups: Control (C), Stress (S), LEO (L) and Stress + LEO (S+L). Immobilization stress was applied to the S groups for 14 days, and LEO was administered to the relevant groups by inhalation. The testicular tissues obtained at the end of the experiment were stained with hematoxylin & eosin and Masson's trichrome for histopathological evaluation. Hematoxylin-eosin-stained testicular sections of all groups were evaluated semi-quantitatively.

Results: That evaluation revealed significant degenerative changes, irregular seminiferous tubules, and interstitial edema in the testicular tissues from the S group. In contrast, the stress-induced damaged tissue in the S + L group exhibited a significant improvement in morphology.

Conclusion: This study demonstrates the potential protective effects of LEO against testicular damage induced by immobilization stress. The administration of LEO partially preserved the structural integrity of the testicular tissue by mitigating histopathological alterations caused by stress. These findings suggest that LEO may be considered as a supportive therapeutic agent against stress-related male reproductive damage.

Keywords: Immobilization stress; lavender essential oil; testis tissue.

Hareketsizlik Stresinin Sıçan Testis Dokusu Üzerindeki Histopatolojik Etkileri ve Lavanta Esansiyel Yağının Koruyucu Rolü

ÖZET

Amaç: Stres çeşitli fizyolojik ve psikolojik bozukluklara yol açabilir ve ayrıca erkek üreme sağlığı üzerinde önemli bir etkiye sahiptir. Stresin kadın doğurganlığı üzerindeki etkileri kapsamlı bir şekilde incelenmiş olsa da, erkek doğurganlığı üzerindeki etkisi nispeten az araştırılmıştır. Bu çalışma, lavanta esansiyel yağının (LEO) sıçanlarda immobilizasyon stresi ile indüklenen testis hasarına karşı potansiyel koruyucu etkisini incelemeyi amaçlamıştır.

Gereç ve Yöntemler: Yirmi dört erkek Wistar Albino sıçanı rastgele dört gruba ayrıldı: Kontrol (C), Stres (S), LEO (L) and Stres + LEO (S+L). Stres gruplarına 14 gün boyunca immobilizasyon stresi uygulandı ve LEO ilgili gruplara inhalasyon yoluyla verildi. Deneyin sonunda elde edilen testis dokuları histopatolojik değerlendirme için hematoksilin ve eozin ve Masson trikromu ile boyandı. Tüm grupların hematoksilin-eozin boyalı testis kesitleri yarı kantitatif olarak değerlendirildi.

Bulgular: Bu değerlendirme, S grubundaki testis dokularında önemli dejeneratif değişiklikler, düzensiz seminifer tübüller ve interstisyel ödem ortaya koydu. Buna karşılık, S + L grubundaki stres kaynaklı hasarlı doku, morfolojide önemli bir iyileşme gösterdi. Bu bulgular, LEO'nun antioksidan ve anti-inflamatuar özellikleri nedeniyle immobilizasyon stres kaynaklı testis hasarını hafifletebileceğini düşündürmektedir.

Sonuç: Bu çalışma, lavanta esansiyel yağının (LEO), immobilizasyon stresi ile indüklenen testiküler hasarı hafifletmede potansiyel koruyucu etkileri olduğunu göstermektedir. LEO uygulaması, stresin neden olduğu histopatolojik bozuklukları azaltarak testis dokusunun yapısal bütünlüğünü kısmen korumuştur. Bu sonuçlar, LEO'nun stres kaynaklı erkek üreme hasarına karşı alternatif bir destekleyici tedavi olarak değerlendirilebileceğini düşündürmektedir.

Anahtar Kelimeler: Hareketsizlik stresi; lavanta yağı; testis dokusu.

1 Adıyaman University, Faculty of Medicine, Department of Histology and Embryology, Türkiye
2 Adıyaman University, Faculty of Medicine, Department of Anatomy, Türkiye

Sorumlu Yazar / Corresponding Author: Elfide Gizem BAKIRHAN, e-mail: ekivrak@adiyaman.edu.tr
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INTRODUCTION

Stress is an inevitable phenomenon in today's world, and one that leads to various physical and psychological problems. Stress factors that disrupt the internal and external environment of the organism and disturb the dynamic state are thought to cause biochemical and functional changes, especially in the endocrine system and nervous system, leading to various complications in the organism and also affecting reproduction (1).

Although the effects of stress on female patients have been well studied, less is understood about the impacts of emotional stress on the male partner. It has been suggested that stress and depression, both commonly encountered phenomena, reduce the levels of testosterone and luteinizing hormone (LH) in the blood, disrupt the functioning of the testis, and thus impairing sperm production and lowering sperm values. Studies have shown that stress impairs fertility, suppresses libido, causes testicular degeneration, and results in deterioration of semen quality (1,2).

The use of alternative and complementary therapies together with modern therapeutic approaches is becoming increasingly popular. Aromatherapy is one such complementary therapy. This involves the application of essential oils as the principal agent in the treatment of various diseases. Essential or volatile oils are obtained from the flowers, stems, leaves, roots, fruits, and other parts of a particular plant through various methods. The principal modes of use include inhalation, local application, and baths. Aromatherapy is used in the treatment of numerous disorders, such as depression, infertility, indigestion, headache, insomnia, muscle pain, respiratory problems, skin diseases, swollen joints, and urinary complications (2). However, rather than relying on the ingestion of various plant-based products, aromatherapy is based on the inhalation of aromas for which therapeutic and psychological properties have been claimed (3).

LEO exerts refreshing and relaxing effects that reduce stress, anxiety, depression, and insomnia (4). There are many types of LEO, the species employed for therapeutic purposes being *Lavandula angustofolia*, or medical LEO. This type grows at higher altitudes, and its therapeutic effects depend on various factors, including even the time when the plant is collected (5). Due to its high levels of therapeutically active compounds, it exhibits numerous health benefits, partly as a result of its antioxidant and anti-inflammatory activities (6). *Lavandula stoechas* essential oils (LEO) have recently been shown to be capable of lowering blood sugar levels and providing protection against alloxan-induced oxidative stress in rats (5).

The modern world contains numerous stress factors. There is therefore an urgent need for a herbal substance capable of both relieving stress and representing an alternative to various drugs that are actively used but whose reliability is controversial. Determining whether medicinal LEO affects testicular tissue in the face of stress and achieving a morphological understanding of possible changes may open a new perspective for future studies on LEO as a potential alternative in the amelioration of stress-related fertility problems.

MATERIAL AND METHODS

The creation of the experimental model

Twenty-four male Wistar Albino rats, 12 weeks of age and weighing 200-250 g, were used in this study. Four groups of six animals were constituted. All experimental animals were obtained from the Adıyaman University Experimental Animals Center following receipt of approval from the Adıyaman University Experimental Animals Ethics Committee (no. 2024/015 dated 02.05.2024), and all experimental procedures were performed in this center according to the principles of the Guide for the Care and Use of Laboratory Animals. The rats were housed in rooms with a room temperature of 22 ± 200 C, in a 12-hour light/dark cycle, and with ad libitum access to food and water throughout the study. The experimental groups were formed as follows;

Control (C): This group was not administered any treatment during the experiment.

Stress (S): The animals in this group were subjected to immobilization stress for 4 hours a day for 14 days (7).

LEO (L): The animals in this group inhaled LEO (%35,67 Linalyl anthranilate, %33,68 Linalool; Producer: Art de Huile, SFA) throughout the experimental period (8).

Stress + LEO (S+L): The animals in this group inhaled LEO and were also exposed to immobilization stress throughout the experiment.

The procedure for applying immobilization stress to the S and S+ L groups was as follows: The animals were immobilized by being placed into a semi-cylindrical acrylic tube (4.5 cm wide and 12 cm long) with suitable holes for breathing. Chronic immobilization stress was applied by keeping them immobilized in this way for specific periods of time during the 14 days study period. The application was carried out for 2 hours in the morning (09:30-11:30) and 2 hours in the afternoon (14:30-16:30). At the end of the experiment, all rats were sacrificed by decapitation. Testicular tissue samples were removed and placed in fixation solutions.

Histological procedures

Following fixation, the testicular tissues were subjected to dehydration and polishing in accordance with routine histological procedures. Next, the tissues were embedded in paraffin blocks, from which 5 µm-thick sections were collected for histological analysis. The sections were then deparaffinized and stained with hematoxylin & eosin (H&E) and Masson's trichrome dye. Histopathological examination was performed under a microscope with a Carl Zeiss digital camera attachment (Carl Zeiss Microscopy GmbH 07745 Jena, Germany).

Histopathological scoring

In this context, 10 different areas in each section were examined under a light microscope using a 10x lens. The possible score range was between 0 and 3, and the sections were scored semi-quantitatively from 0 to 3. According to this scoring, the absence of pathology was scored as 0, the presence of mild pathology as 1, the presence of moderate pathology as 2, and the presence of severe pathology as 3 (9). Quantitative results were obtained by making comparisons between groups. All parameters were evaluated independently by an expert histologist who was unaware of the study groups.

Statistical Analyses

Numerical data were analyzed using SPSS software (SPSS version 21.0; SPSS Inc., Chicago, IL, USA) and expressed as mean ± standard deviation. Normality (Shapiro-Wilk) and homogeneity tests confirmed that all four groups followed a normal distribution. Differences between groups were compared using one-way ANOVA. Tukey's post hoc test was applied for multiple comparisons. The results were evaluated within a 95% confidence interval, and a p-value of <0.05 was considered statistically significant.

RESULTS

Histopathological Findings

Histopathological evaluation of the C and L groups revealed a normal histological architecture in the seminiferous tubules, seminiferous epithelial cells, and interstitial connective tissue. The spermatogonia, primary spermatocytes, and spermatids that constituted the spermatogenetic series were regularly arranged on the walls of the seminiferous tubules. No pathology was observed in the evaluation (Figures C1, C2 -L1, L2). Histopathological evaluation of the S group revealed significant morphological changes compared with the C and L groups. Degeneration was observed in the seminiferous epithelium, together with irregular seminiferous tubule shapes, and edema findings in the interstitial connective tissue (Figures S1-3). Examination of the S+ L revealed a similar structure to those in the C and L groups due to the protective effect of LEO. Seminiferous tubule regularity was preserved, and a decrease was determined in the interstitial connective tissue edema. Some signs of degeneration were present in the seminiferous epithelium (Figures S+L1-2). The tissue samples from each group were surrounded by the tunica albuginea, dense connective tissue (Figures C3-S3-L3-S+L3).

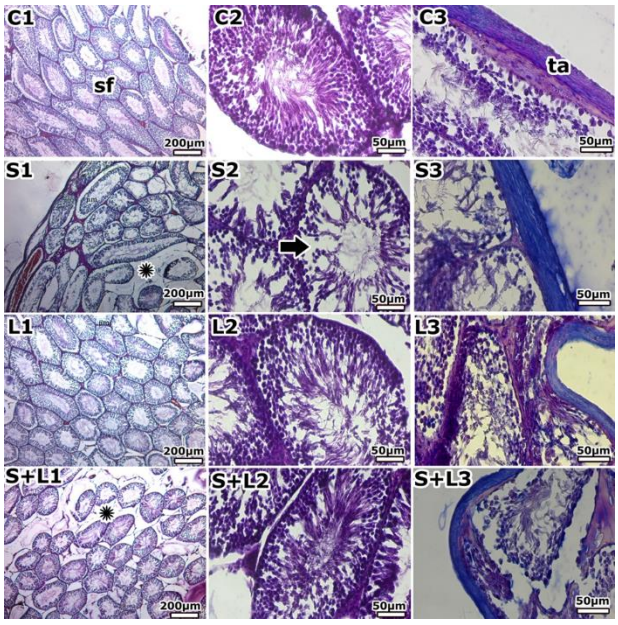

Figure 1. (C1-3): Images of testicular tissue from the C group, (S1-3): Images from the S group, (L1-3): Images from the L group, (S+L1-3): Images from the S + L group. (C1, S1, L1 and S+L1; x10 objective lens magnification, C2-3, S2-3, L2-3 and S+L2-3 and x40 objective lens magnification; 1 and 2 H&E staining, 3 Masson's trichrome staining) sf; seminiferous tubules, ta; connective tissue sheath, arrow; seminiferous epithelial degeneration, star; edema in the interstitial connective tissue area.

Histopathological scoring

When hematoxylin-eosin-stained testicular sections of all groups were evaluated semi-quantitatively in terms of degeneration in the seminiferous epithelium and irregular shapes of the seminiferous tubules, no statistically significant difference was found between C, S, L and S+L groups (p>0.05) (Fig. 2, 4). When the groups were compared in terms of edema formation in the interstitial connective tissue, it was seen that edema formation increased in S group compared to C group (p<0.01) (Fig. 3). Similarly, the increase in edema in S group compared to S+L group was statistically significant (Fig.3). On the other hand, there was no statistically significant difference between S and L groups (p<0.05) (Fig.3).

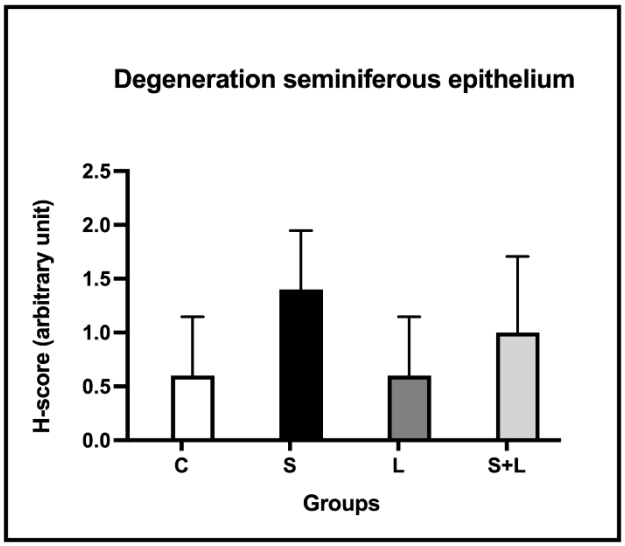

Figure 2. Semi-quantitative evaluation of degeneration in the seminiferous epithelium parameter in all groups (mean±SD).

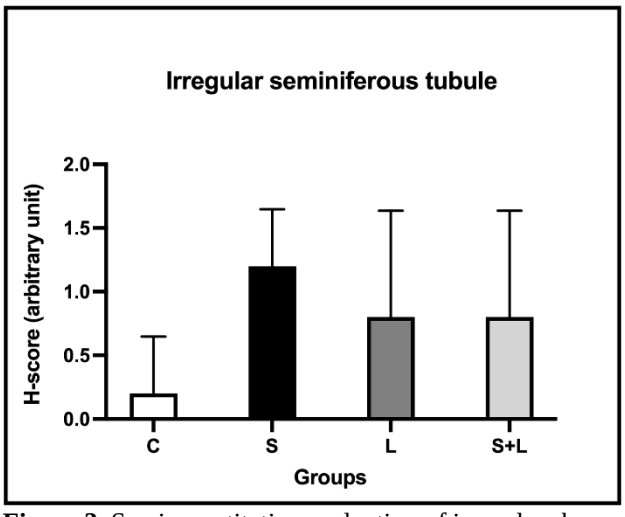

Figure 3. Semi-quantitative evaluation of irregular shapes of the seminiferous tubules' parameter in all groups (mean±SD).

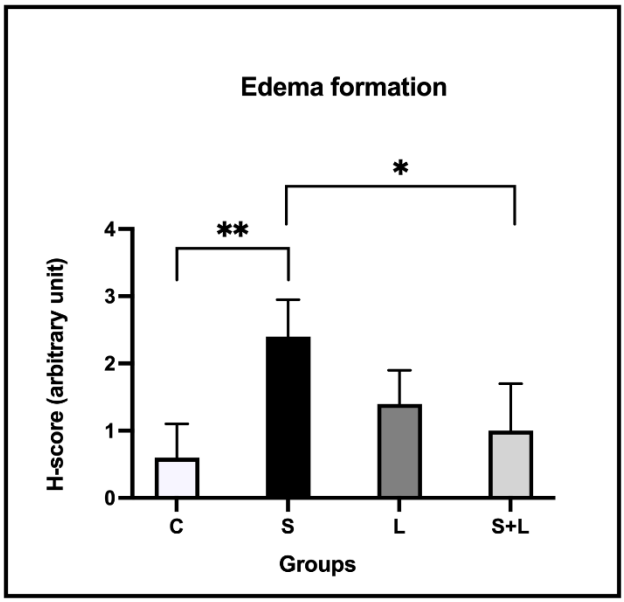

Figure 4. Semi-quantitative evaluation of edema formation in the interstitial connective tissue parameter in all groups. Differences at the level of $p<0.05$ were shown with "*" while differences at the level of $p<0.01$were pointed out by "**" (mean±SD).

DISCUSSION

The rise in human infertility that may result from physiological and psychological stress has recently attracted considerable attention. Although stress-induced testicular dysfunction and the pathological characteristics thereof have been well described in rats, the animal models used are unsatisfactory and the results are contradictory. In the present study, the adverse effects of immobilization stress on male infertility in adult rats were evaluated histopathologically, and the potential protective role of LEO was investigated on the basis of its antioxidant activities. The findings revealed that stress caused significant damage to the testicular architecture, while the application of LEO significantly alleviated this.

Experimental studies attempt to understand the effects of stress on human tissues by exposing animals to different stresses. Several studies have observed deleterious effects on the hormonal system and reproductive health. In the current study, Wistar Albino rats were subjected to immobilization in order to mimic physiological and psychological stress. The immobilization stress model is generally regarded as easily implemented and effective.

Various studies have shown that stress causes free radical formation and oxidative tissue damage. Immobilization stress disrupts the body's antioxidant balance by causing overproduction of reactive oxygen species (ROS). The organic balance between ROS and antioxidants is highly significant in normal sperm physiological processes such as capacitation, hyperactivation, acrosome reactions, and signaling processes needed to achieve fertilization (10). When the antioxidant defense mechanism is disabled, sperm functions deteriorate significantly. These disturbances cause peroxidative damage to the sperm plasma membrane, extensive damage to DNA, proteins and lipids, and male infertility through mechanisms that include apoptosis (11). Karna et al. (2020) examined testicular dysfunction due to immobilization stress and reported that this induces oxidative stress by reducing testicular superoxide dismutase, glutathione peroxidase (GPx), glutathione peroxidase-4 (GPx 4), catalase, nuclear factor erythroid 2-related factor 2, and heme oxygenase 1) levels and increasing those of malondialdehyde (MDA) and ROS/reactive nitrogen species. However, they observed that oxidative stress induced by immobilization stress led to an absence of spermatozoa, irregular seminiferous tubules, and vacuolated seminiferous tubules exhibiting degeneration in testicular tissues (12). However, another study exposed animals to restraining stress for seven days and then examined their testicular tissues. Histological examination revealed a low sperm mass in the epididymal lumen and some atrophy in the seminiferous tubules (13). The results of the present study are consistent with this literature. The group exposed to stress only exhibited notable pathological changes, such as irregularities in seminiferous tubules, degeneration in epithelial tissue, and edema between tissues. This suggests that stress disrupts the antioxidant defense system, adversely impacts affects sperm production, and causes deterioration in testicular structure.

LEO is a natural ingredient with a long history of use in traditional and complementary medicine. Known for its antioxidant, anti-inflammatory and relaxing effects, LEO also provided remarkable protection in this study (14,15). No deleterious findings in testicular tissue were observed in the group given LEO. Seminiferous tubule disorders and edema, especially in the S group, can be regarded as clear indicators of testicular damage, LEO application significantly alleviated these findings. LEO possesses the capacity to scavenge free radicals such as hydroxyl radical (OH°), the main cause of lipid peroxidation, due to the phenolic compounds it contains. Sperm cell membranes are rich in polyunsaturated fatty acids and are highly susceptible to free radical attacks. Lipid peroxidation of sperm cell membranes is therefore a form of cell damage induced by ROS. A previous study reported that LEO played a potential protective role against the decrease in sperm quality and sperm motility resulting from the increase in ROS induced by malathion (1). Similar effects have been reported in previous studies. Researchers have reported that LEO reduces oxidative damage and preserves the body's natural balance (16,17). Hamidi et al. (2015) investigated the protective role of LEO in impaired spermatogenesis caused by cadmium chloride in rats and observed that it significantly reduced the increased MDA levels caused by cadmium chloride exposure. They also concluded that it regulated total antioxidants, LH, and testosterone levels, and improved semen parameters impaired by cadmium chloride (18). When all these findings are evaluated together, it may be concluded that LEO significantly alleviates histopathological damage caused by chronic stress in testicular tissue due to its antioxidant and cell protective effects. LEO's capacity to scavenge free radicals and its potential to protect sperm membranes against oxidative stress suggest that it may have positive effects on male reproductive health. In line with the previous literature, this study is consistent with the protective effect of LEO on testicular structure. However, this effect can be demonstrated more comprehensively with future studies at both experimental and clinical levels. In conclusion, LEO can be considered

as a protective support element against reproductive system disorders caused by immobilization stress.

In our study, the absence of biochemical evaluation of oxidative stress markers constitutes one of its limitations. Oxidative stress markers such as malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), and glutathione peroxidase (GPx) are critically important for confirming the presence and extent of oxidative damage and antioxidant defense, particularly in stress-related studies. The inclusion of such parameters could have strengthened the interpretation of the antioxidant potential of LEO and its protective mechanism against testicular damage. Therefore, future studies should incorporate comprehensive biochemical assessments alongside histological and immunohistochemical evaluations to better elucidate the relevant molecular pathways.

CONCLUSION

Modern life can produce a constant state of stress, which is capable of affecting numerous systems, including male reproductive health. The data from this study show that LEO can play a supportive role in reducing these stress-related damages. We think that such natural support may become increasingly important, especially in the context of complementary methods such as aromatherapy. However, more extensive studies, supported at the molecular level and involving different doses and application times, are now needed in order to establish whether this effect also applies to humans.

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