

Gallik Asitin Hareketsizlik Stresinin Erkek Üreme Sisteminde Oluşturduğu Hasar Üzerine Antioksidan Etkisi

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ÖZET

Giriş ve Amaç: Bu çalışmada, gallik asidin stres kaynaklı testis hasarına karşı olası koruyucu etkisi ile oksidatif stres belirteçleri üzerindeki rolü araştırılmıştır.

Gereç ve Yöntemler: Toplam 28 adet erkek Sprague-Dawley sıçan dört gruba ayrıldı: Kontrol (C), Gallik Asit (GA), Kronik Stres (CS) ve Kronik Stres + Gallik Asit (CS+GA). CS grubuna, 15 gün boyunca her gün 6 saat süren hareketsizlik stresi uygulandı. GA grubuna 15 gün boyunca günde 20 mg/kg gallik asit oral yolla verildi. CS+GA grubuna gallik asit, stres protokolünden hemen önce verildi. Histolojik, immünohistokimyasal ve biyokimyasal analizler yapıldı ve elde edilen veriler SPSS programı kullanılarak analiz edildi.

Bulgular: Kontrol ve GA gruplarında normal testis morfolojisi gözlemlendi. CS grubunda, seminifer tübüllerde bozulma ve oksidatif stres belirteçlerinde artış saptandı. CS+GA grubunda ise bu hasarın azaldığı ve kan-testis bariyerinin bütünlüğünün kısmen korunduğu tespit edildi.

Sonuç: Gallik asit, stres kaynaklı testis hasarını azaltmada koruyucu etki göstermiştir. Bu etkisiyle, nedeni açıklanamayan erkek infertilitesine yönelik destekleyici bir ajan olarak potansiyel taşıyabilir.

Anahtar Kelimeler: Erkek İnfertilitesi, Hareketsizlik Stresi, Oksidatif Stres, Antioksidan, Gallik Asit

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The Antioxidant Effect Of Gallic Acid On The Damage Caused By Immobilization Stress To The Male Reproductive System

ABSTRACT

Aim: To assess the potential of gallic acid in stress-induced testicular damage and its impact on oxidative stress markers

Material and methods: Twenty-eight male Sprague-Dawley rats were divided into four groups: Control (C), Gallic Acid (GA), Chronic Stress (CS), and Chronic Stress + Gallic Acid (CS+GA). The CS group experienced 6-hour immobilization stress for 15 days, while the GA group received 20 mg/kg oral gallic acid daily for 15 days. The CS+GA group received gallic acid before exposure to chronic immobility stress.

Histological, immunohistochemical and biochemical analyses were conducted, and data were analyzed using the SPSS program.

Results: Normal testicular morphology was observed in the Control and Gallic Acid groups. The Chronic Stress group exhibited impaired seminiferous tubules and increased oxidative stress markers. However, damage decreased in the Chronic Stress + Gallic Acid group, showing restored blood-testis barrier integrity.

Conclusion: Gallic acid demonstrated potential in reducing stress-induced testicular damage, suggesting its potential as a supportive agent in male infertility of unknown etiology.

Keywords: *Male Infertility, Immobilization Stress, Oxidative Stress, Antioxidant, Gallic Acid*

1. INTRODUCTION

Stress, encompassing both physiological and psychological aspects, is a common condition that adversely affects the daily functioning of all organisms (Konturek et al., 2011). Prolonged stress conditions disturb the crucial state of homeostasis necessary for the maintenance of life across all systems of an organism (Cetinel et al., 2005). The stress arising from societal and economic pressures in daily life is believed to underlie numerous diseases (Cikler et al., 2005). To explore the impact of stressors encountered in daily life, such as economic and social pressures linked to human depression, on male sexual behavior, investigations have utilized the immobilization stress model in animal studies (Mustafa et al., 2019). Research findings have indicated that immobilization stress leads to an elevated incidence of abnormal sperm, degeneration within the seminiferous tubules, and a reduction in male reproductive capacity (Ribeiro et al., 2018). Utilizing animal models in research holds significant importance in uncovering disease causation and evaluating the efficacy of therapeutic interventions (Fuchs & Flugge, 2006).

Oxidative stress is defined as the inability of endogenous antioxidant defense mechanisms to counteract elevated levels of oxidative substances induced by internal and external stressors. Prolonged exposure to oxidative stress ultimately leads to cellular death (M. B. AYCAN Et Al.). Chronic inflammation, a protracted immune response, stands as a significant contributor to oxidative stress, elevating the levels of reactive oxygen species (ROS) (Poritz et al., 2007). Intracellular antioxidant systems are responsible for averting

ROS formation under normal physiological conditions or eliminating free oxygen radicals and their precursors (Arai et al., 1989). Exogenous supplementation through antioxidants markedly reduces tissue damage caused by oxidative stress (Sogut et al., 2015). These defense systems exhibit their efficacy by scavenging ROS, inhibiting their generation, or amending resultant damage (Lobo et al., 2010; Moreno et al., 2005). Antioxidants are categorized into natural antioxidants, synthesized within the body, such as superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) (Moreno et al., 2005). In instances where endogenous antioxidants are insufficient, derived natural antioxidant compounds administered in medicinal forms can be employed (M. B. AYCAN Et Al.). Certain medicinal plants possess the capacity to ameliorate damage caused by chronic stress by restoring oxidative stress markers closer to normal levels. Gallic acid (GA), recognized as a phenolic compound present in natural sources, demonstrates promising antioxidant attributes. Studies have indicated its capacity for antioxidant and anti-inflammatory effects. The phenolic hydroxyl groups within gallic acid exhibit antioxidative actions by scavenging reactive oxygen species (ROS) and reducing lipid peroxidation owing to their redox characteristics (Rajan & Muraleedharan, 2017).

In our investigation, testicular injury was induced through chronic immobilization stress, serving as an experimental model simulating daily-life stress. We explored the histological and biochemical effects of gallic acid, assessing its potential to mitigate this damage owing to its recognized antioxidant and anti-inflammatory properties. Our findings suggest that gallic acid may offer a supplementary therapeutic agent for addressing male infertility of undetermined origin, postulated to be linked with stress-induced factors.

2. METHOD

2.1 Animals

A total of 28 male Sprague-Dawley rats with an average weight of 200–250 g were used in the experiments. The subjects were kept in standard cages with a 12-hour light/dark cycle, 60% humidity and 22–23 °C temperature 1 week before the experiments began and during the 15-day experimental protocol. The feed and water needs of the subjects were met with standard pellet feed and ad libitum drinking water. Ethical approval for all procedures performed on the animals was obtained from the Health Sciences University Hamidiye Experimental Animals Local Ethics Committee (15.06.2022-06-01). The experiments and animal care were conducted at the Hamidiye Experimental Animal Production and Research Laboratory of the University of Health Sciences.

2.2 Experimental Groups

A cohort of 28 male Sprague-Dawley rats was allocated into four distinct groups, each consisting of seven subjects:

Control (C): No intervention was administered (n=7)

Gallic Acid (GA): Animals received a daily oral gavage of 20 mg/kg gallic acid for a duration of 15 days (n=7)(Ramkumar et al., 2014)

Chronic Stress (CS): Animals were exposed to 6 hours of daily immobilization stress for a period of 15 days (n=7)(Smith, 2012)

Chronic Stress+Gallic Acid (CS+GA): Animals received 20 mg/kg gallic acid via oral gavage before each daily 6-hour immobilization stress session over a 15-day period (n=7)

2.3 Experimental Protocol

The experimental protocol involved utilizing 15x5x5 sized plexiglass stress mechanisms to implement the immobilization stress model. Rats were secured by their tails within these mechanisms and subjected to immobilization stress consistently at the same time daily (09:00-15:00) for a duration of 15 days. Freshly prepared gallic acid (Sigma, Germany), dissolved in distilled water (pH≈6), was orally administered to subjects in the GA and CS+GA groups at a dosage of 20 mg/kg per rat each day. No additional solvents or buffers were used.

2.4 Histological Preparation

The testicular samples were fixed in Bouin's solution for standard histological analysis. Following a 48-hour fixation period, the testis underwent dehydration in a series of increasing alcohol concentrations, starting from 70% alcohol with lithium carbonate. Subsequently, they were made transparent and embedded in paraffin blocks. Sections of approximately 5 µm thickness were obtained using a MICROM HM325 microtome from Thermo Scientific, Germany. The histological assessment included examination of general testicular structure through hematoxylin-eosin (H-E) staining, assessment of basement membrane thickness via Periodic Acid-Schiff (PAS) reaction, and visualization of mast cell morphology in the tissue using toluidine blue (TB) staining. Microscopic examination of all sections was conducted using a microscope equipped with a Zeiss AxioCam camera attachment.

2.5 Immunohistochemical Preparation

The testicular specimens designated for ZO-1 immunohistochemistry were fixed in NBF (neutral buffered formalin) for 24 hours. Sections averaging 5 µm in thickness were obtained from paraffin-embedded testis samples. These sections underwent deparaffinization using xylene, followed by dehydration in a series of decreasing alcohol concentrations, and were subsequently treated in citrate buffer (pH: 6, DIAPATH Lot: 2019x05445). After treatment with 3% hydrogen peroxide (H₂O₂), they were incubated with the primary ZO-1 antibody (Abcam, ab221547). The subsequent step involved horseradish peroxidase (HRP) treatment, followed by incubation with the secondary antibody (Abcam, ab64264 Lot: GR3461544-1). Sections were counterstained using diaminobenzidine (DAB) and hematoxylin, underwent a series of increasing alcohol concentrations, and were finally sealed with entellan (Merck, GERMANY Lot: HX90503161). For the evaluation of ZO-1 immunostaining, 20 tubules were randomly selected from each testicular section. Scoring was based on both the intensity and the

prevalence of staining. The staining was graded as follows: 4 for intense and diffuse staining, 3 for moderately intense and diffuse staining, 2 for slight and uncommon staining, 1 for minimal staining, and 0 for no detectable staining (Acikel-Elmas et al., 2023; Kırmızıkan et al., 2025).

2.6 Morphometric Evaluation

For each subject, 100 seminiferous tubules were randomly selected. Photographs of each selected seminiferous tubule were taken at x50 microscope magnification, and the inner and outer diameters and basement membrane thickness of the seminiferous tubules were measured on the photo (Zen Blue-Edition Prg). To evaluate spermatogenesis, 100 seminiferous tubules randomly selected from each section were given a score between 0 and 10 in accordance with the Johnsen scoring system (Johnsen, 1970).

2.7 Biochemical Evaluation

Testis samples were homogenized by weighing (QIAGEN TissueLyser LT, Hilden, Germany). Total protein determination was measured at 595 nm using a commercial kit based on the Bradford method (Coomassie Plus, Protein Assay, Thermofisher Scientific, Massachusetts, USA). The Bradford protein test was used to determine the total protein content in the tissue (Bradford, 1976). Oxidative Stress Index (OSI) value was found by calculating the Total Oxidant Status (TOS) /Total Antioxidant Status (TAS) ratio of the groups.

2.8 Statistical Analyses

Descriptive statistics for the quantitative variables in the study are presented as mean and standard deviation. The normality of distribution was assessed using the Shapiro-Wilk test. Homogeneity of variances was tested using Levene's test. To evaluate the mean differences between groups, Brown-Forsythe and one-way analysis of variance (ANOVA) were used. For multiple comparisons, post hoc tests including Dunnett T3 and Tukey tests were employed. The statistical significance level was set at 0.05, and the calculations were performed using IBM SPSS (Version 26.0. Armonk, NY: IBM Corp.) software.

3. RESULTS

The findings of this study suggest that gallic acid may exert protective effects against stress-induced testicular damage through its antioxidant and anti-inflammatory properties. Further studies are needed to confirm its potential role in the management of male infertility.

Table 1. ZO-1 immunohistochemical staining scores of the experimental groups. The CS group showed a statistically significant decrease compared to the Control and GA groups (** $p < 0.001$), while the CS+GA group showed a significant increase compared to the CS group (** $p < 0.001$), though it remained lower than the Control group.

Groups	ZO-1 Score
C	3.58 ± 0.61
GA	3.63 ± 0.54
CS	2.06 ± 0.45 ***
CS+GA	2.98 ± 0.49 ***

3.1 Light Microscopy

In the histological examination using H-E and PAS staining of testicular sections from the C and GA groups, the seminiferous tubules displayed healthy architecture with well-aligned cellular structures. Abundant sperm were observed within the tubular lumen. The basement membrane appeared smooth and continuous, and the interstitial area maintained its structural integrity. Conversely, in the CS group, notable disruptions and losses in spermatogenic cell series were evident, leading to compromised tubular morphology. Reduced sperm count was observed within the tubular lumen compared to the C and GA groups. However, in the CS+GA group, the seminiferous tubule morphology resembled that of the C and GA groups, displaying well-organized spermatogenic series. Sperm count within the lumen increased compared to the C group. Moreover, the interstitial tissue, which exhibited structural deterioration in the CS group, regained its integrity akin to the C and GA groups (Figure 1a, b, c, d and figure 1e, f, g, h).

In sections stained with toluidine blue, assessment of mast cell morphology within the interstitial area beneath the tunica albuginea was conducted. The C and GA groups displayed a limited number of granulated mast cells in this region. Conversely, in the CS group, an increase in mast cell numbers was observed both under the tunica albuginea and around blood vessels, including granulated and degranulated mast cells. In contrast, the CS+GA group exhibited a reduction in granulated mast cell count beneath the tunica albuginea compared to the C group. These mast cells were primarily located near a few damaged tubules (Figure 1 j, k, m, n).

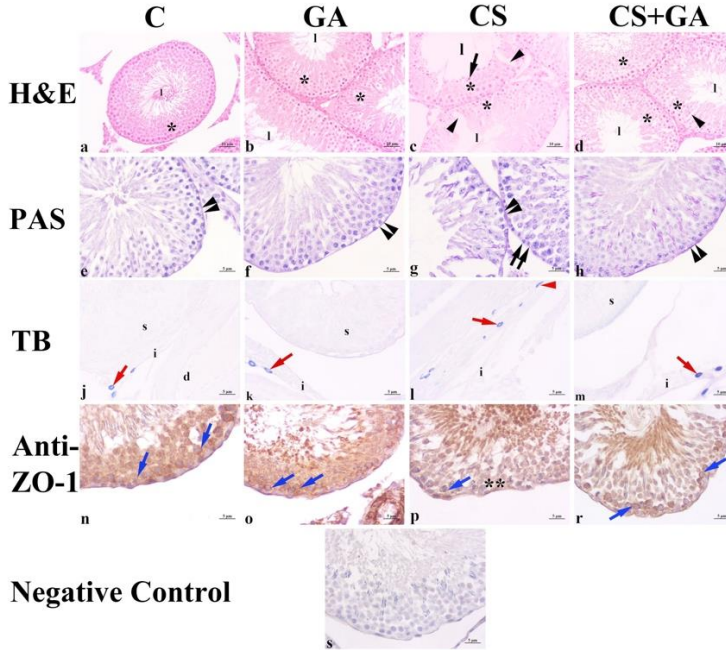


Figure 1. Light microscopic and immunohistochemical comparison of experimental groups. (a–d) Hematoxylin and eosin (H&E) staining of testicular tissue sections ($\times 200$). Germinal epithelium (*), lumen (L), separation areas in the germinal epithelium (arrows), and disruptions within the germinal epithelium (arrowheads) are shown.

(e–h) Periodic acid-Schiff (PAS) staining ($\times 400$). Basement membrane (double arrowheads); disrupted basement membrane continuity (double arrows) is observed, particularly in the CS group.

(j–m) Toluidine blue staining ($\times 400$). Seminiferous tubule (s), interstitial area (i), blood vessel (d), granular mast cells (red arrows), and degranulated mast cells (red arrowheads) are indicated.

(n–r) Immunohistochemical staining for ZO-1 ($\times 400$). Positive immunoreactivity of the blood-testis barrier (blue arrows) is visible. Disruption in basal localization of the barrier is marked (asterisk) in the CS group.

(s) Negative control section for ZO-1, showing no specific immunoreactivity.

3.2 Immunohistochemistry

Immunohistochemical analysis revealed a continuous ZO-1 expression marking at the level of the blood-testis barrier in the C group. Conversely, in the CS group, areas with germinal epithelial losses exhibited an absence of ZO-1 markings. In regions without epithelial loss, ZO-1 immunolabeling appeared weak and sporadic. Quantitative scoring demonstrated a

significant reduction in ZO-1 expression in the CS group compared to the control and GA groups (** $p < 0.001$).

However, in the CS+GA group, ZO-1 positive markings were evident within the germinal epithelium, showcasing a substantial reduction in disruptions observed at the blood-testis barrier compared to the CS group. The semiquantitative analysis further confirmed that the ZO-1 immunoreactivity in the CS+GA group was significantly higher than in the CS group (** $p < 0.001$), although it remained lower than the control group (** $p < 0.001$) (Table 1). Specificity of ZO-1 labeling was confirmed using negative control sections processed without primary antibody (Figure 1p-u).

3.3 Morphometry

Based on morphometric assessments, the seminiferous tubule diameter and germinal epithelial thickness exhibited a decrease, while the basement membrane thickness showed an increase in the CS group compared to the C group. Conversely, in the CS+GA group, these parameters approached values similar to those observed in the C group (Figure 2 1a, b, and c).

Regarding Johnsen scoring, a significant decrease in score values was noted in the CS group compared to the C group (** $p < 0.01$). However, in the CS+GA group, the score values resembled those of the C group. Moreover, the score values significantly increased in the CS+GA group compared to the CS group and were similar to values observed in the C and GA groups (** $p < 0.01$) (Table 2).

Table 2. Johnsen Score values of the experimental groups. The CS group showed a statistically significant decrease compared to the Control and GA groups (** $p < 0.01$), while the CS+GA group showed a significant increase compared to the CS group (** $p < 0.01$), reaching values similar to the Control group.

Groups	Johnsen Score
C	9±0.5
GA	9.04±0.35
CS	7.68±0.27**
CS+GA	8.86±0.59**

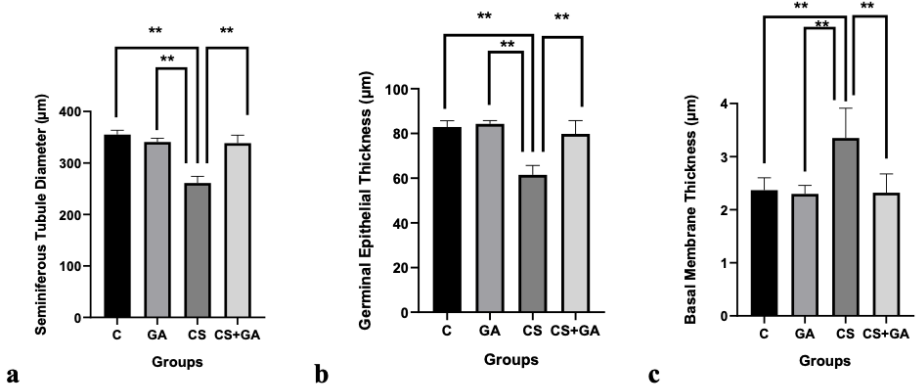


Figure 2. Comparison of seminiferous tubule diameters, germinal epithelium thickness, and basal membrane thickness between experimental groups. (a) Comparison of seminiferous tubule diameters, (b) Comparison of germinal epithelium thickness, and (c) Comparison of basal membrane thickness. (*p<0.05), (**p<0.01)

3.4 Biochemical Results

The TAS levels measured within testicular tissue exhibited a notable decrease in the CS group compared to the C group (**p<0.01). Although there was an increase in TAS values in the CS+GA group in comparison to the CS group, this difference did not reach statistical significance. Conversely, the TOS values displayed a significant increase in the CS group compared to the C group (*p<0.05). However, in the CS+GA group, TOS values significantly decreased when compared to the CS group (*p<0.05). The OSI values demonstrated a significant rise in the CS group compared to the C group (*p<0.05), and notably decreased in the CS+GA group compared to the C group (Table 3).

Table 3. TAS, TOS, and OSI values measured in testicular tissues. The CS group showed a significant increase in TOS and OSI values compared to the Control group (*p<0.05), while the CS+GA group showed significantly lower TOS and OSI values compared to the CS group (*p<0.05). No statistically significant difference was observed in TAS values between the groups.

Groups	TAS	TOS	OSI
C	0.026±0.004	0.164±0.013	6.427±1.186
GA	0.034±0.006	0.128±0.024	3.768±0.528
CS	0.197±0.002	0.227±0.028*	11.538±1.439*
CS+GA	0.022±0.004	0.180±0.042*	8.428±2.513*

4. DISCUSSION

Stress represents a natural adaptive reaction of an organism to disturbances that challenge its internal balance (Chrousos, 2009). In the modern era, the increasing variety and intensity of stressors are recognized as significant factors adversely affecting human health (Chudzik). Infertility, an issue affecting approximately one in eight couples of reproductive age, often remains unexplained, with stress speculated to contribute to cases of unknown etiology (Hamada et al., 2012). Defined as the failure to conceive after a year or more of regular unprotected intercourse, infertility involves male-related factors in approximately 50% of cases, with 37–58% of cases having no identifiable cause (Marin et al., 2007).

Upon exposure to stressors, the activation of the hypothalamo-pituitary-adrenal axis (HPA) is an initial response in living organisms (Aitken et al., 2014). While this activation initially serves as a natural adaptation to maintain organismal balance, chronic activation leads to elevated cortisol levels—its end product—triggering an upsurge in inflammatory responses and the generation of reactive oxygen species (ROS) within tissues. The heightened ROS levels in the male reproductive system are implicated in oxidative stress-induced damage, particularly affecting cells within the seminiferous tubules (Acevedo-Rodriguez et al., 2018).

Excessive stimulation of the HPA axis due to chronic stress also influences the activity of the hypothalamo-pituitary-gonadal (HPG) axis. The HPG axis plays a crucial role in testicular function, and disruptions in this axis lead to diminished testicular volume and reduced testosterone levels (Oyola & Handa, 2017). Al-Damegh et al. reported a decline in testosterone levels following 6 hours of immobility stress in their study (Al-Damegh, 2014). In our research, although the difference was not statistically significant, we observed a tendency toward reduced serum testosterone levels in the chronic stress group exposed to immobilization stress compared to the control group. Regarding oxidative stress, we noted a tendency toward decreased Total Antioxidant Status (TAS) levels and significantly elevated Total Oxidant Status (TOS) levels in the chronic stress group compared to the control and gallic acid groups. These alterations in oxidative stress markers suggest an increase in oxidative stress within tissues and inadequacy in endogenous antioxidant systems due to chronic stress. The decline in serum testosterone levels, alongside heightened oxidative stress in testicular tissue due to immobility stress, indicates disruption in the functioning of the HPG axis. Elevated oxidative stress within the tissue also triggers cellular morphological damage.

Joo et al. previously reported a decrease in seminiferous tubule diameters, thickening of the basement membrane, and a reduction in the Johnsen biopsy score caused by chronic immobility stress in their study examining the damage to the male reproductive system (Joo et al., 2006). Similarly, our study observed diminished seminiferous tubule diameters, germinal epithelial losses, and basement membrane thickening in the chronic stress group. Furthermore, our evaluation of the Johnsen score in the same group compared to the control

and gallic acid groups revealed similar findings. These observations indicate that chronic immobility stress induces morphological damage in the testicles alongside increased oxidative stress, resulting in decreased serum testosterone levels according to our biochemical assessments. The seminiferous tubules, responsible for housing male reproductive cells and enabling spermatogenesis, may suffer cell losses and damage, potentially disrupting the spermatogenesis process and contributing to male infertility.

Under stress conditions, the accumulation of ROS in tissues intensifies inflammation. This inflammatory response correlates with an increase in mast cell migration and activation (Esposito et al., 2002). Active mast cells, via the secretion of pro-inflammatory cytokines, contribute to the inflammatory response by enhancing vascular permeability and participating in fibrotic processes. Studies have linked active mast cells to stimulating collagen synthesis in infertile men (Cardoso et al., 2019). In our investigation, we evaluated testicular tissues for mast cell morphology and collagen-rich seminiferous tubule basal membranes. We noted a significant increase in active mast cells and pronounced thickening of seminiferous tubule basement membranes in the chronic stress group compared to the control and gallic acid groups. This membrane thickening is likely attributable to activated mast cells stimulating collagen deposition.

Elevated oxidative stress within the tissue leads to detrimental effects on cell membranes, protein constituents, and DNA within the seminiferous tubules, contributing to cell loss. The impairment of intercellular connections in cell membranes not only affects spermatogenic cells but also compromises the Sertoli cells, crucial for maintaining the blood-testis barrier's integrity, thereby safeguarding the process of spermatogenesis. Disruption of this barrier among Sertoli cells results in the loss of spermatogenic cells and the generation of a reduced number of impaired sperm (Zhou et al., 2020).

ZO-1 protein plays a pivotal role in forming the blood-testis barrier by facilitating the adhesion of integral membrane proteins such as occludin and claudin to the actin cell skeleton (Hervé et al., 2014). Studies, like the one conducted by Musch et al., have indicated that stress negatively impacts ZO-1 protein and impairs the stability of these connections (Musch et al., 2006). In our investigation, anti-ZO-1 immunohistochemistry revealed a significant decrease in ZO-1 marked areas within the seminiferous tubules of the chronic stress group, displaying notably weakened reactions. These findings underscore how chronic stress disrupts the structural proteins involved in the blood-testis barrier among Sertoli cells. Consequently, it diminishes the protective environment necessary for the survival and completion of spermatogenesis for the limited number of surviving germ cells affected by the compromised blood-testis barrier.

In the male reproductive system, a harmonious equilibrium between ROS and the internal defense mechanism of antioxidants is vital. Optimal ROS levels are crucial for the normal functionality of newly generated sperm. However, due to their inherently low antioxidant capacity, sperm cells are particularly susceptible to oxidative stress (Cardoso et al., 2019). When oxidative stress intensifies beyond manageable levels, the inadequacy of internal

antioxidant defenses disrupts the equilibrium. This disruption leads to structural damage within the seminiferous tubules, affecting Sertoli and germ cells, consequently impeding the proper progression of spermatogenesis. Deficient and faulty spermatogenesis results in male infertility. The incorporation of external antioxidant supplements is highly beneficial in reducing tissue damage induced by oxidative stress (Agarwal et al., 2004; Cardoso et al., 2019). Behairy et al. demonstrated in their research that supplementation with vitamin C effectively reduced oxidative damage in the testis of male rats (Behairy et al., 2020).

Gallic acid, a recognized compound abundant in plants, possesses well-documented antioxidant and anti-inflammatory attributes owing to its phenolic nature. Its phenolic hydroxyl groups actively combat ROS and inhibit lipid peroxidation through their redox properties (Rajan & Muraleedharan, 2017). Olukole et al. reported that a daily administration of 20 mg/kg GA exhibited protective effects against testicular damage induced by bisphenol (Olukole et al., 2020). Additionally, Chhillar et al. highlighted GA's antidepressant properties when administered at 20 mg/kg per day (Chhillar & Dhingra, 2013).

In our investigation, the application of GA alongside chronic stress demonstrated an elevation in serum testosterone levels compared to the chronic stress group. These findings suggest that GA, by exerting antioxidant effects, preserves the functionality of both the HPA and HPG axes, consequently elevating testosterone levels—known to decrease under stress—nearly to control levels. Moreover, our study revealed a reduction in TOS levels within the chronic stress group when GA was administered. Concurrently, TAS, initially low, displayed an increase. This observation suggests that GA, leveraging its antioxidant properties, reduces oxidative stress and aids in reinstating homeostasis by complementing diminished endogenous antioxidants within the testicles.

Oxidative stress is known to inflict damage on tissues and cells, inciting heightened inflammation by activating mast cells (Theoharides & Cochrane, 2004). GA owing to its antioxidant and anti-inflammatory attributes, is anticipated to mitigate oxidative stress and inflammation within testicular tissues. Indeed, our study's examination of mast cell morphology unveiled a noticeable decrease in mast cell numbers and activity within the chronic stress group treated with GA compared to the group subjected solely to chronic stress. By neutralizing ROS and curbing inflammation in the testicles, the damage induced by stress on cells was markedly decreased. Our comprehensive morphological assessments corroborated this assertion. The CS+GA group exhibited increased diameters of seminiferous tubules, largely preserved intratubular epithelial integrity, heightened epithelial height, and a notable presence of sperm displaying within the tubule lumen. Furthermore, evaluation of seminiferous tubule basement membranes in the same group indicated a reduction in thickness compared to the chronic stress group. This reduction suggests that the diminished number and activity of mast cells, observed in the CS+GA, induce less stimulation of fibrosis.

In the group subjected to chronic stress alongside GA treatment, the integrity of the blood-testis barrier between neighboring Sertoli cells within the seminiferous tubules remained notably sustained. This observation aligned with the reduced cellular damage and inflammation observed compared to the chronic stress group. Notably, sections from the CS+GA displayed more pronounced immune labeling at the intercellular junctions evaluated through anti-ZO-1 immunostaining compared to the chronic stress group. Consequently, the utilization of GA seemed to mitigate oxidative stress-induced damage to cell membranes and proteins by reducing ROS.

Our findings highlight the potential role of gallic acid, known for its antidepressant, antioxidant, and anti-inflammatory properties, in mitigating the decline in testosterone levels resulting from chronic immobilization stress. Additionally, GA demonstrated efficacy in diminishing cellular damage within testicular tissues, stemming from heightened oxidative stress, thus contributing to tissue integrity preservation. Moreover, GA exhibited an anti-inflammatory effect, reducing fibrosis in the thickened seminiferous tubule basement membranes, nearly restoring them to control levels. This anti-inflammatory action significantly minimized tissue damage. By supporting the integrity of the blood-testis barrier, GA may have contributed to the maintenance of spermatogenesis.

Statement of Research and Publication Ethics

All processes of the study were conducted in accordance with the research and publication ethics principles of the Journal of Health Sciences Institute of Manisa Celal Bayar University.

Author Contributions

The first author contributed 30%, the 2nd, 3rd, 4th, and 5th authors each contributed 10%, and the 6th author contributed 30% to the manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest with any individual or institution.

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