ÖZGÜN ARAŞTIRMA ORIGINAL RESEARCH

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INVESTIGATION OF THE EFFECT OF OLANZAPINE AND L-CARNITINE ON RAT TESTIS TISSUE

OLANZAPİN VE L-KARNİTİNİN RAT TESTİS DOKUSU ÜZERİNDEKİ ETKİSİNİN ARAŞTIRILMASI

Merve AKKUŞ[1], Faruk KILIÇ[2], Halil AŞÇI[3], Fatma Nihan CANKARA[3], Mehtap SAVRAN[3], Dilek ULUSOY KARATOPUK[4]

[1] Kütahya Health Sciences University, Faculty of Medicine, Department of Psychiatry, Kütahya, TÜRKİYE
[2] Süleyman Demirel University, Faculty of Medicine, Department of Psychiatry, Isparta, TÜRKİYE
[3] Süleyman Demirel University, Faculty of Medicine, Department of Pharmacology, Isparta, TÜRKİYE
[4] Suleyman Demirel University, Faculty of Medicine, Department of Histology and Embryology, Isparta, TÜRKİYE

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Öz

Amaç
Olanzapinin cinsel işlev bozukluğu üzerine olumsuz etkileri vardır. Üreme sisteminde de yapısal değişikliklere neden olduğunu gösteren sınırlı sayıda çalışma bulunmaktadır. Bu çalışmada Olanzapinin neden olduğu testis hasarı üzerine L-Karnitin'in etkilerinin histopatolojik, sperm parametreleri, biyokimyasal açıdan incelenmesi amaçlanmıştır.

Gereç ve Yöntem
Çalışmamızda toplam 48 adet erişkin Sprague-Dawley erkek sıçan kullanıldı. Sıçanlar, her grupta 8 sıçan olacak şekilde 6 gruba ayrıldı: Kontrol grubu (C), 200 mg/kg L-Karnitin uygulanan grup (LC), 2 mg/kg düşük doz Olanzapin verilen grup (LOZN), 2 mg/kg Olanzapin ve 200 mg/kg L-Karnitin verilen grup (LOZN+LC), 4 mg/kg Olanzapin verilen grup (HOZN), 4 mg/kg Olanzapin ve 200 mg/kg L-Karnitin verilen grup (HOZN+LC). MDA, IL-1B, IL-6, TAS, TOS düzeylerinin belirlenmesi, histolojik değerlendirme için testis dokuları kullanıldı.

Bulgular
Olanzapin alan gruplarda sperm sayısında azalma, MDA, IL-1β, TOS değerlerinde artış, testis dokusunda doza bağlı histopatolojik değişiklikler gözlendi. Olanzapin ve L-Karnitin uygulanan gruplarda sadece Olanzapin verilen gruplara göre histopatolojik değişiklikler daha düşük oranda bulundu.

Sonuç
Yüksek doz Olanzapin uygulanan gruplarda gözlenen testis yapısındaki dejeneratif histolojik bulguların, Olanzapinin testis dokusunda oluşturduğu oksidatif stresten kaynaklanabileceği sonucuna varıldı. L-Karnitin ise oksidatif hasarı azaltarak testis dejenerasyonun azaltılmasında etkili olabileceği düşünüldü.

Anahtar Kelimeler: L-Karnitin, Oksidatif stres, Olanzapin, Sperm, Testis

Abstract

Objective
Olanzapine has adverse effects on sexual dysfunction. There are a limited number of studies showing that it

Sorumlu yazar ve iletişim adresi / Corresponding author and contact address: M.A. / merveorhanakkus@gmail.com
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ORCID IDs of the authors:M.A: 0000-0003-3046-2815; F.K:0000-0002-2259-4028; H.A: 0000-0002-3677-5567; F.N.C: 0000-0002-2367-6412; M.S: 0000-0002-7933-0453; D.U.K: 0000-0002-9984-294X

also causes structural changes in the reproductive system. This study aimed to examine the effects of L-Carnitine on testicular damage caused by Olanzapine in terms of histopathological, sperm parameters, and biochemical aspects.

Material and Method
A total of 48 adult Sprague-Dawley male rats were used in our study. Rats were divided into 6 groups, 8 rats in each group: the Control group (C), 200 mg/kg L-Carnitine administered group (LC), 2 mg/kg low dose Olanzapine administered group (LOZN), 2 mg/kg Olanzapine, and 200 mg/kg L-Carnitine administered group (LOZN+LC), 4 mg/kg Olanzapine administered group (HOZN), 4 mg/kg Olanzapine and 200 mg/kg L-Carnitine administered group (HOZN+LC). Testicular tissues were used for the determination of MDA, IL-1B, IL-6, TAS, TOS levels, and histological evaluation.

Results
In the groups receiving Olanzapine, a decrease in sperm count, an increase in MDA, IL-1β, TOS values, and dose-dependent histopathological changes in testicular tissue were observed. Histopathological changes were found at a lower rate in the Olanzapine and L-Carnitine administered groups compared to the Olanzapine-only groups.

Conclusion
It was concluded that the degenerative histological findings in the testicular structure observed in the high-dose Olanzapine administered groups might be caused by the oxidative stress induced by Olanzapine in the testicular tissue. L-Carnitine, on the other hand, was thought to be effective in reducing testicular degeneration by reducing oxidative damage.

Keywords: L-Carnitine, Olanzapine, Oxidative stress, Sperm, Testis

Introduction

Environmental toxicants, occupational exposures, and drug-induced adverse reproductive effects are important indicators of male infertility. The male reproductive system may be the target of drug toxicity. Continuous exposure to antipsychotic drugs alters hypothalamic-pituitary and gonadal hormones or non-hormonal mechanisms in men, leading to impaired sexual function, spermatogenesis process, as well as epididymal maturation (1). Olanzapine is atypical, in other words, a second-generation antipsychotic that acts through dopamine and serotonin receptors (2). There are studies in the literature that Olanzapine is associated with male infertility through sexual dysfunction (3, 4).

Oxidative stress is an imbalance between the formation of Reactive Oxygen Species (ROS) and antioxidant defense mechanisms (5). This imbalance leads to damage to biomolecules and cells that are vital to the whole organism. ROS are products of normal cellular metabolism and play an essential role in stimulating signaling pathways in animal cells in response to changes in intracellular and extracellular environmental conditions (6). Oxidative stress can seriously damage proteins, lipids, DNA, and organelles. It is a process directly related to inflammation and causes the secretion of many cytokines and chemokines in inflammatory cells (7).

Oxidative stress can activate various transcription factors leading to differential expression of some genes involved in inflammatory pathways. Inflammation triggered by oxidative stress is the cause of many chronic diseases (8).

Inflammation is an adaptive response to harmful conditions, despite the diversity of inflammatory phenomena (9). To restore body homeostasis, the inflammation process must be strictly controlled, and its termination must also be in a controlled manner. Therefore, the activation of inflammatory cells, recruitment, and modulation of migrating inflammatory cells must be stopped. When acute neutralization fails inflammation occurs, there is a risk of developing chronic inflammation and can result in inflammation leading to adverse metabolic effects. (10).

L-Carnitine is a biologically active stereoisomer of 3-carboxy-2-hydroxy-N, N, N-trimethyl-1-propanamide. L-Carnitine has been shown to have beneficial effects on parameters such as motility, maturation, and fertilization capacity in male germ cell lines through its antioxidant and radical scavenging properties (11-13). L-Carnitine has significant effects on the male reproductive system, notably on sperm count and motility (14). Spermatozoa are immobile when they reach the epididymis. As it passes through the epididymis, as a result of post-gonadal modification with an increase in L-Carnitine in the epididymal fluid, spermatozoa mature, and tail movement is observed (15). L-Carnitine is known to be effective in improving fertilization ability with post-gonadal modification (16).

Due to the severe side effects of Olanzapine, especially its sexual dysfunction and adverse effects on the reproductive system, drug use is often interrupted. It is aimed to investigate the role of L-Carnitine in Olanzapine-induced testicular damage, which has limited studies on structural changes in the reproductive system.

Material and Method

Animal experiments were approved by the Local Animal Ethics Committee of Süleyman Demirel University (Ethics No: 14-03, dated 13.04.2018). 48 adult male Spraque-Dawley rats weighing 280-300 g were housed at 21-22 °C and 60% ± 5% humidity, with a 12-hour light, 12-hour dark cycle. All rats were fed with standard commercial chow (Korkuteli Yem, Antalya, Turkey), ad libitum food, and water.

They were divided into 6 groups with 8 animals in each group. Olanzapine doses were determined as 2 and 4 mg/kg, which is equivalent to the high doses used in humans (0.5 and 2.5 mg/kg/day) according to the literature review (2, 17, 18).

Olanzapine (from Abdi İbrahim, Istanbul, Turkey) was dissolved in distilled water and administered by oral gavage to experimental animals. The duration of administration was determined as 42 days, based on repeated dose oral toxicity studies and literature review, during which possible reproductive system damage could be detected (19, 20).

Based on the literature review, the dose of L-Carnitine (from World Medicine, Istanbul, Turkey) administered to the experimental animals was determined as 200 mg/kg/day as an intraperitoneal injection (21, 22).

Control group (Control) (n=8): For 6 weeks, i.p. normal saline (SF) and oral gavage were applied in the same injection volume as the other groups.

Low-dose olanzapine group (LOZN) (n=8): Olanzapine was administered by oral gavage at a dose of 2 mg/kg/day for 6 weeks.

High-dose olanzapine (HOZN) (n=8): Olanzapine was administered by oral gavage at a dose of 4 mg/kg/day for 6 weeks.

Low dose olanzapine + L-Carnitine (LOZN + LC) (n=8): Olanzapine + 200 mg/kg i.p. L-Carnitine was administered by oral gavage at a dose of 2 mg/kg/day for 6 weeks.

High-dose olanzapine + L-Carnitine (HOZN+LC) (n=8): Olanzapine at a 4 mg/kg/day dose by oral gavage + 200 mg/kg i.p. L-Carnitine was administered for 6 weeks.

L-Carnitine (LC) (n=8): 200 mg/kg i.p. L-Carnitine was administered for 6 weeks.

The weights of the rats in the experimental groups were measured before the first drug administration and 24 hours after the last administration. During the experiment, the weighing process was repeated weakly. At the end of the 6 weeks, rats that were given ketamine/xylazine (80-100 mg/kg-6-8 mg/kg) anesthesia i.p. were sacrificed by collecting a high amount of blood from the vena cava inferior.

Histopathological Analyses

In the histopathological examinations, the tissues were washed in running water overnight using the immersion fixation method in 10% neutral formalin solution, and then they were subjected to dehydration, clearing, infiltration, and embedding processes. Sections of 4-micron thickness were taken from the prepared paraffin blocks using a Leica-type slide microtome. Preparations were stained with Hematoxylin-Eosin for histological evaluation. The stained samples were examined under an Olympus BX50-type binocular microscope, and images were obtained and evaluated.

Isolated epididymides were kept in Petri dishes with 2 ml of stock solution. For the evaluation of epididymal spermatozoa, the epididymides, which were divided into two, were placed in a stock solution with a Petri dish. It was kept at 37°C for 10-15 minutes to float and separated into 1 ml Eppendorf tubes. Sperm samples were evaluated after vortexing. They were evaluated with the Olympus CX21 (40X) stereomicroscope and Makler camera at 36.5ºC. All groups were kept confidential and scored by the same person by making five different counts of each sample. For total motility, five different samples were prepared for each group. A minimum of 200-300 sperm samples were counted for optimization. Progressive motility was determined as (a+b), non-progressive (c), and immotile as (d). Total motility was determined as (a+b) based on WHO 2010.

Biochemical Analyses

Collected blood samples were kept at 4 C for 24 hours and then centrifuged at 2500 rpm for 15 minutes, and the serum fractions were used to determine serum FSH, LH, and testosterone levels based on the experimental procedure determined by the manufacturer of the relevant kits. Rat testis and

epididymis tissues were removed and washed with phosphate buffer (8 g/L NaCl, 0.224 g/L KCl, 0.2 g/L KH_2PO_4, 1.14 g/L Na_2HPO_4, pH 7.4) to remove blood and contamination. Left testicular tissues were used to determine the levels of testicular malondialdehyde (MDA), Interleukin-1 beta (IL-1B), Interleukin-6 (IL-6), Total Antioxidant Status (TAS), Total Oxidant Status (TOS), and Oxidative Stress Index (OSI) based on the experimental procedure determined by the manufacturer of the respective kits.

Some of the left testicular tissues were used for biochemical analyses. After weighing the tissue samples, they were homogenized in 750 L PBS in a homogenizer (Tissuelyser II, QIAGEN, Germany) for about 3 minutes at 30 frequencies. During the homogenization processes, care was taken to keep the samples under cold conditions. The homogenate was centrifuged at 10,000 g, and the resulting supernatants were stored at -80˚C until analysis. MDA, IL-1B, IL-6, TAS, and TOS levels were measured from the supernatants as an indicator of oxidative stress.

Statistical Analyses

Statistical analyzes were performed using the SPSS 20.0 package program. A value of $p<0.05$ was accepted as the cut-off value of statistical significance. Results were expressed as mean±standard deviation. One-Way Analysis of Variance (ANOVA) was used to compare the groups' body weight, sperm parameters, and blood values. When a significant difference was detected, Tukey or Tamhane posthoc tests were used by examining the homogeneity status of group variances to determine between which groups the difference was. Kruskal-Wallis test was used to compare tissue parameters, and when a significant difference was detected, pairwise comparison tests were used to determine between which groups the difference was. A chi-square test was used to compare sperm morphological parameters.

Results

In our study, one animal from each C and LOZN group died before the experiment was terminated. Therefore, the experimental findings were interpreted on 46 animals.

Evaluation of Animal Weights

When the first and sixth-week weight average of the rats, which were measured every week for six weeks, was evaluated, statistically significant weight loss was observed only in the LC group ($t=3.904$, $p=0.006$). No significant weight change was observed in the other groups ($p>0.05$).

Evaluation of Biochemical and Hormonal Parameters in Testicular Tissue

A statistically significant difference was found in MDA, IL-1B, and TOS values, the parameters measured in testicular tissue ($F=12.277$, $p=0.031$, $F=14.561$, $p=0.012$, $F=16.342$, $p=0.006$, respectively). There was no statistically significant difference in IL-6, TAS, and OSI values ($p>0.05$).

When the MDA values were examined, there were statistically significant differences between Control and LOZN (LOZN>C, $p=0.031$), Control and

Table 1 MDA, IL-1B, IL-6, TAS, TOS, OSI Values of the Groups

	Controls (n=7)	LC (n=8)	LOZN (n=7)	LOZN+LC (n=8)	HOZN (n=8)	HOZN+LC (n=8)	Test statistic	P
MDA (nmol/g)	0.909±0.158	1.107±0.114	1.122±0.113	1.225±0.161	1.006±0.283	1.137±0.133	12,277	**0.031***
IL-1B (pg/mL)	1.388±0.189	1.268±0.481	1.664±0.174	1.629±0.124	1.415±0.241	1.379±0.196	14,742	**0.012***
IL-6 (pg/mL)	1.387±0.115	1.418±0.125	1.368±0.122	1.419±0.104	1.335±0.111	1.323±0.163	3,420	0.636
TAS (mmolTrolox Equivalent/L)	1.288±0.323	1.890±1.212	1.734 ±1.207	1.350±0.861	0.983±1.163	1.550 ±1.073	4,115	0.533
TOS (µmol H2O2 Equivalent/ L)	26.386±4.292	25.320±6.311	33.590±12.076	22.531±3.30	31.999±8.579	22.106±2.136	16,342	**0.006***
OSI	2.157±0.623	1.751±0.888	2.496±1.519	2.069±2.150	4.160±5.584	2.340±1.735	3,372	0.643

MDA: Malondialdehyde; IL-1B: Interleukin-1 beta; IL-6: Interleukin-6; TAS: Total Antioxidant Status; TOS: Total Oxidant Status; OSI: Oxidative Stress Index; LC: L-Carnitine group; LOZN: Low-dose Olanzapine group; LOZN+LC: Low-dose Olanzapine+L-Carnitine group; HOZN: High-dose Olanzapine group; HOZN+LC: High-dose Olanzapine+L-carnitine group; Kruskal-Wallis test

LOZN+LC (LOZN+LC>C, p=0.001), Control and HOZN+LC (HOZN+LC>C, p=0.019), HOZN and LOZN+LC groups (LOZN+LC>HOZN, p=0.04).

When the IL-1β values were examined, there were statistically significant differences between the Control and LOZN (LOZN>Control, p=0.028), Control and LOZN+LC (LOZN+LC>C, p=0.035), LC and LOZN (LOZN>LC, p=0.011), LC and LOZN+LC (LOZN+LC>LC, p=0.014), HOZN and LOZN (LOZN>HOZN, p=0.032), HOZN+LC and LOZN (LOZN>HOZN+LC, p=0.009), HOZN+LC and LOZN+LC groups (LOZN+LC>HOZN+LC, p=0.012).

When the TOS values were examined, there was a statistically significant difference between the groups HOZN+LC and LOZN (LOZN>HOZN+LC, p=0.003), HOZN and HOZN+LC (HOZN>HOZN+LC, p=0.005), LC and LOZN (LOZN>LC, p=0.049), LOZN and LOZN+LC (LOZN>LOZN+LC, p=0.005), and HOZN and LOZN+LC groups (HOZN>LOZN+LC, p=0.010).

No statistically significant difference was found between the groups in terms of blood FSH, LH, and testosterone levels in our study (p>0.05) (Table 1).

Evaluation of Sperm Counts of Groups

The graph shows the sperm counts of all groups evaluated at the end of the six-week experimental protocol. When evaluated in terms of sperm counts, there is a statistically significant difference (F=4.195 p=0.004). As a result of posthoc analysis, the sperm count of the L-Carnitine applied group was found to be significantly higher than the HOZN and LOZN groups (p=0.01, p=0.033, respectively). No statistically significant difference was observed between the other groups (p>0.05).

When evaluated in terms of sperm motility, there was no statistically significant difference between the groups (F=1.678, p=0.163)(Figure 1).

Evaluation of Sperm Morphologies

As a result of the evaluation of sperm morphologies, significantly higher abnormal morphology was observed in the HOZN group than in all other groups. Significantly higher abnormal morphology was observed in the HOZN+LC group than in the control group (Figure 2).

In the groups receiving olanzapine, the percentage of sperm with abnormal morphology increased dose-dependently, which was most observed in the HOZN group. The percentage of abnormal morphology decreased in the olanzapine and L-Carnitine administered groups.

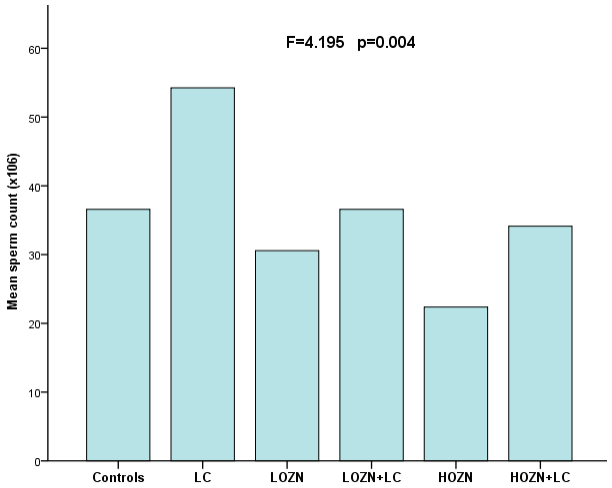

Figure 1
Comparison of Average Sperm Counts Between Groups

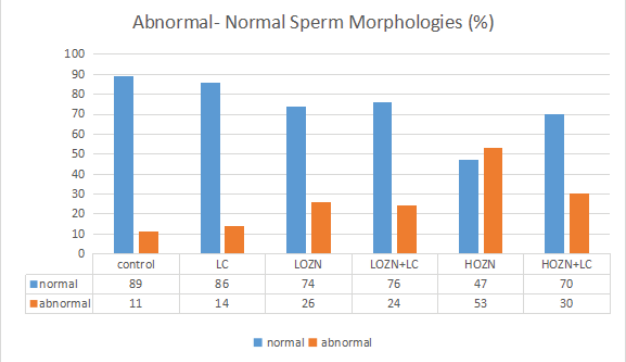

Figure 2
Distribution of Sperm Rates with Abnormal and Normal Morphology by Groups

When the morphological abnormalities were evaluated by classifying them as head, neck, tail, and cytoplasmic residues, it was observed that the cytoplasmic residue ratio increased concerning the Olanzapine dose, which was the highest in the HOZN group, and this ratio decreased with the addition of L-Carnitine to the treatment. When evaluated in terms of head, neck, and tail abnormalities, a higher rate of abnormalities was observed in the Olanzapine-treated groups compared to the control and L-Carnitine groups.

Histopathological Findings

Testicular samples of all groups in our study were observed in terms of the basement membrane, seminiferous tubule structures, interstitial space structures, and cells cytoplasm and nuclei by light

microscopic examination. The samples in the control group were observed to be normal in terms of the examined areas. In the light microscopic examination of LC group testis samples, it was observed that it maintained its normal appearance in the interstitial area, seminiferous tubule structure, and lumen. It was also observed that spermatogenesis continued.

In the light microscopic examination of rat testis samples in the LOZN group, the basement membrane, seminiferous tubule structures, cytoplasm and nuclei of Leydig cells in the interstitial area, vascular structures, Sertoli cells, spermatogonia, and spermatogenic series cells were observed in a smooth structure, while edema was observed in the interstitial area. Spermatogonia were localized between Sertoli cells and were observed in the tail lumen towards the head tubule wall.

Light microscopic examinations of rat testis samples from the LOZN+LC group were similar to the control and L-Carnitine groups. There was no interstitial edema observed in the LOZN group. It was observed that the seminiferous tubule structures were normal, with a normal histological appearance in terms of interstitial area features and cells.

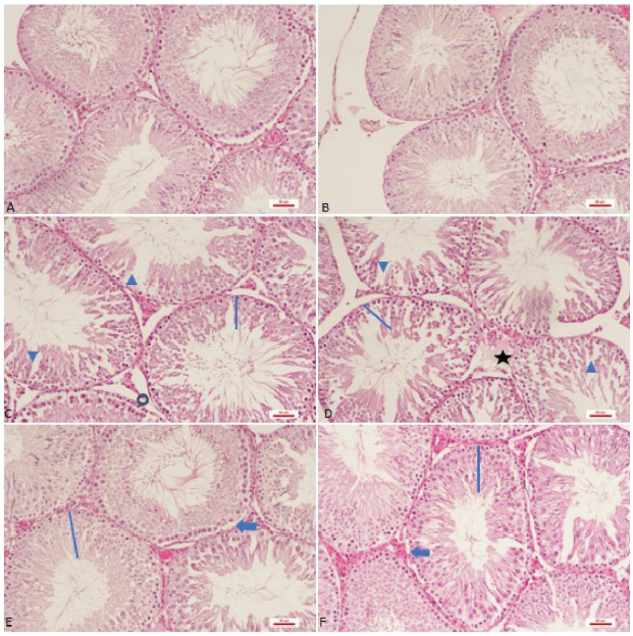

Figure 3
Comparison of Intergroup Histopathological Results

In the light microscopic examination of rat testis samples from the HOZN group, occasional deteriorations and losses in the basal membrane seminiferous tubule structures, and congestion and edema in the interstitial structure were observed. Some openings in the seminiferous tubule lumens and occasional losses in the interstitial areas were observed. Organizational disorders were occasionally observed in the germinal epithelium. Deterioration and decrease in spermatogenic series in cells were noted. In the light microscopic examination of rat testis tissue samples from the HOZN+LC group, features similar to the HOZN group were observed in the basal membrane and seminiferous tubule structures. It was observed that there were improvements (decreases) in the losses in the interstitial areas in the opening of the seminiferous tubules compared to the HOZN group. It has been understood that the degeneration in the cells continues with the decrease (Figure 3).

Discussion

The effectiveness of antipsychotic drugs is due to their effects on the dopaminergic system, which plays a role in the regulation of emotional life, control of motivation, modulation of perceptions, and regulation of behavior. In this study, the effects of L-Carnitine on histopathological, oxidative stress, and hormone parameters in testis damage induced by Olanzapine were evaluated.

In control, LC, and LOZN+LC groups, normal histological appearance regarding seminiferous tubule structures, interstitial area features, and cells were detected. In the LOZN group, although normal histological appearance was observed in terms of seminiferous tubule structures and cells, edema was observed in the interstitial area. In the HOZN group, histopathological changes such as openings of the seminiferous tubule lumens, losses in the interstitial areas, organizational disorders in the germinal epithelium, and degeneration of the cells were observed. Among the findings we obtained in our study, the opening of the seminiferous tubule lumens, losses in the interstitial areas, organizational disorders in the germinal epithelium, and degeneration in the cells observed in the HOZN group may be considered an indicator of testicular toxicity. The histopathological changes observed in our study indicate that Olanzapine may have dose-dependent toxicity on the testicular tissue since these changes were detected in the HOZN-administered group, and the histopathological appearance of the LOZN group was similar to the control group in terms of other features, together with edema in the interstitial area. The fact that degenerative changes were seen at a lower rate in the HOZN+LC group compared to the HOZN group indicates that L-Carnitine administration causes a regression in the degenerative effect in the testicular tissue.

The effects of Olanzapine on testicular histology have been demonstrated with few studies in the literature (19, 24). In the study of De Siqueira Bringel et al., germ cell desquamation, multinucleated giant cells, vacuolization in Sertoli cells, necrotic and apoptotic germ cells were observed in rats treated with 5 and 10 mg/kg Olanzapine (19). These histopathological changes observed, parallel to our study, show that Olanzapine has degenerative effects on testicular tissue, and this effect is dose-dependent. In the study of Soliman et al., it was stated that it caused epithelial desquamation, epithelial detachment, and apoptotic changes in the germ cells of the rat testis in the group receiving Olanzapine, and vacuolization, endoplasmic reticulum dilatation, and lipid accumulation were shown in Sertoli cells (24). Unlike our study, they detected histopathological changes in the testis with a lower dose (0.5 mg/kg/day) of Olanzapine administration. This effect was thought to be related to the use of Olanzapine for a longer period (14 weeks), even at low doses.

The positive effects of L-Carnitine on testicular tissue and the male reproductive system have been shown in many studies (25- 28). In the study of Deliktas et al., the protective effect of L-Carnitine on ischemia and reperfusion injury caused by testicular torsion was investigated. Histologically, degeneration of germinal epithelial cells, edema of interstitial tissue, and color changes in spermatocytes were observed after ischemia-reperfusion. After administration of L-Carnitine, histological improvement was found close to normal (25). In the study of Khushboo et al., it was shown that the harmful effects of long-term copper consumption on sperm quality and testicular function were improved with L-Carnitine. L-Carnitine therapy has been shown to protect tubules effectively by significantly reducing seminiferous tubule damage (28). It is known that L-Carnitine has positive effects on the male reproductive system, especially with its antioxidant and radical scavenging properties, on sperm maturation, motility, and fertilization capacity (11, 12). In our study, we found that L-Carnitine administration caused a regression in the degenerative effect in the testicular tissue, in line with the literature.

There was no dose-dependent statistical difference in sperm concentration in the olanzapine-treated groups. This finding is consistent with the literature. In the study conducted by De Siqueira Bringel et al., no statistically significant difference was found between the experimental groups regarding sperm count of rats administered i.p. 1-2.5-5-10 mg/kg Olanzapine for 45 days (19). When the groups were compared in terms of sperm morphology, it was seen that the proportion of sperm with abnormal morphology increased in a dose-dependent manner in the groups receiving Olanzapine; however, administration of L-Carnitine with Olanzapine caused a decrease in abnormal morphology. Significantly higher abnormal morphology was observed in the HOZN group than in all other groups. When the morphological abnormalities were evaluated by classifying them as head, neck, tail, and cytoplasmic residues, it was observed that the cytoplasmic residue ratio increased concerning the Olanzapine dose and was highest in the HOZN group, and this ratio decreased with the administration of L-Carnitine. When evaluated in terms of head, neck, and tail abnormalities, a higher rate of abnormality was observed in the other groups than in the Control and LC groups. These findings show that Olanzapine may negatively affect sperm morphology, and the addition of L-Carnitine may reduce abnormal morphology.

Atypical antipsychotic drugs such as Olanzapine have serious side effects such as weight gain and metabolic dysfunction (31). In our study, statistically significant weight loss was observed only in the L-Carnitine applied group. No significant weight change was observed in the other groups. Unlike the clinical situation, it has been reported that Olanzapine-induced weight gain in rats causes more weight gain in female rats depending on gender (32). The effects of L-Carnitine on weight reduction have been reported in the literature (33). As a result of the data, we obtained in our study, the absence of weight gain in the rats administered Olanzapine and the decrease in body weight in the group administered L-Carnitine is compatible with the literature. In the study of Davey et al., when the body weights of male and female rats given Olanzapine (2 and 4 mg/kg/day) for 3 weeks were compared, significant body weight gain was observed only in female rats. It was thought that gender had a role in the course of some side effects of antipsychotics and that body weight gain in female rats was associated with hyperphagia (34). Weight gain is observed significantly with the use of Olanzapine in clinical practice. However, in rat studies in the literature, it was reported that this finding was not observed in male rats, as in our study. The mechanism of this situation has not been fully clarified.

It is stated that ROS is found at a high rate in the semen analysis of infertile men (35). In our study, a significant difference was found in MDA, IL-1 β, and TOS values, which are the parameters measured in testicular tissue. Considering the MDA values, a significant difference was found between the Control

and LOZN, Control and HOZN+LC, Control and LOZN+LC groups. In our study, it was thought that the increase in MDA levels compared to the control group might be an indicator of increased oxidative stress associated with Olanzapine.

It has been reported in the literature that olanzapine increases IL-1β levels (36). Consistent with the literature, we also found that IL-1β levels increased with olanzapine in our study. We may suggest that Olanzapine may cause an increase in inflammation. When the TOS values are examined, there is a significant difference between groups. The higher TOS values in the Olanzapine administered groups, the increase in Olanzapine-related oxidative stress, and the lower TOS values in the Olanzapine and L-Carnitine applied groups were interpreted as the antioxidant effect of L-Carnitine. Eftekhari et al. evaluated the cytotoxic effect of Olanzapine on rat hepatocytes in their study. Olanzapine cytotoxicity in hepatocytes was found to be associated with ROS, potential mitochondrial collapse, increase in lysosomal membrane permeability, and could be statistically significantly inhibited by ROS scavengers, antioxidants, and endocytosis inhibitors (37).

In the study of Bilgic et al., in which the kidney damage and metabolic effects of Olanzapine were examined, they found statistically significant changes in TAS, TOS, and OSI levels and stated that oxidative stress played a role in Olanzapine-induced nephrotoxicity (38). In the study of Togar et al., in which they investigated the genotoxic and oxidative damage potential of Olanzapine in vitro, it was shown that Olanzapine induced oxidative stress due to high doses. It has been stated that tissue damage due to oxidative stress may occur (39). Many studies in the literature have stated that testicular degeneration can develop due to many reasons, and oxidative stress is also effective in these degenerative processes (40- 42). In the study conducted by Ahmed et al. investigating the protective role of L-Carnitine against testicular damage due to γ-ray radiation, it was shown that TNF-α, IL1-β, and IFN-γ mRNA expressions were decreased in the L-Carnitine applied group compared to the γ-ray irradiation group (43). The increase in reactive oxygen species in semen causes lipid oxidation, loss of membrane integrity, and an increase in permeability. It has been reported to cause decreased sperm count and activity, motility, and abnormal morphology as a result of the inactivation of cellular enzymes, structural DNA damage, and cell apoptosis (44, 45).

Many of the antipsychotics block dopamine (D2) in the central nervous system, leading to an increase in prolactin levels and thus suppression of the hypothalamic-pituitary-gonadal axis, which may cause a decrease in GnRH levels and a secondary decrease in serum LH, FSH, and testosterone levels (46, 47). Conflicting results were reported in studies conducted with Olanzapine in rats and examining reproductive hormone levels (12, 47). Yanik et al. (47) found no significant difference in testosterone and LH levels in rats given Olanzapine (4mg/kg/day) and risperidone (2mg/kg/day), but a significant decrease in FSH levels. On the other hand, De Siqueira Bringel et al. found an 84% decrease in plasma testosterone levels in rats given 10 mg/kg olanzapine, while they did not detect a significant difference in plasma testosterone levels in rats given 2.5 and 5 mg/kg Olanzapine (19). No significant difference was found between the groups regarding the blood FSH, LH, and testosterone levels in our study. Considering our study and other studies, we think that the reason for this is that the dose we choose may not be sufficient for significant changes in hormones.

Some results were obtained in our study. First, considering the histopathological changes, the opening of the seminiferous tubule lumens, losses in the interstitial areas, organizational disorders in the germinal epithelium, and degeneration of the cells may be an indicator of the testicular toxicity of Olanzapine. The milder findings in the LOZN group and the more severe degenerative findings in the HOZN and HOZN+LC groups suggest that this effect is dose-dependent. Olanzapine-induced testicular degenerative changes were observed less in the HOZN+LC group than in the HOZN group, indicating that L-Carnitine may positively affect Olanzapine-induced testicular degeneration. Second, the decrease in sperm count due to the damage caused by Olanzapine was reversed with L-Carnitine treatment, resulting in a significant increase in sperm count. Third, it was concluded that abnormal sperm morphology and degenerative histological findings in testicular structure in high-dose Olanzapine administered groups might be associated with Olanzapine-induced oxidative stress in testicular tissue. Following the literature, it was concluded that L-Carnitine may have a positive effect on sperm morphology and testicular degeneration by reducing oxidative damage.

The limitation of the study is the inability to perform immunostaining at the tissue level beyond histopathological imaging. Immunostaining could not be performed due to the thesis work and the use of a limited budget. However, in light of the findings of our study, it is noteworthy that there is a limited

number of studies showing that Olanzapine causes histopathological changes in testicular tissue, the mechanisms of these changes have not yet been clarified, and studies suggesting that Olanzapine has effects on oxidative stress in other organs. Our study is the first to investigate the effects of Olanzapine and L-Carnitine on testicular tissue through oxidative stress parameters. Further animal and human studies are required to fully comprehend the effects of Olanzapine and L-Carnitine on testicular tissue.

Conflict of Interest Statement
The authors have no conflicts of interest to declare.

Ethical Approval
Animal experiments were approved by the Local Animal Ethics Committee of Süleyman Demirel University (Ethics No: 14-03, dated 13.04.2018).

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Data available on request from the authors.

Authors Contributions
MA: Conceptualization, Data curation, Formal analysis, Writing-original draft.

FK: Conceptualization, Formal analysis, Funding acquisition, Project administration, Supervision, Writing-review & editing.

HA: Conceptualization, Data curation, Investigation, Methodology, Resources

FNC: Statistical analysis, Formal analysis, Investigation, Methodology

MS: Data curation, Investigation, Methodology, Writing-review & editing.

DUK: Formal analysis, Resources, Visualization

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