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RESEARCH ARTICLE

How are testicular functions affected after morphine dependence and withdrawal in rats?

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ABSTRACT

Objective: The effects of morphine addiction on testicular activity are significant. This study aimed to examine testicular contraction and tissue changes that may occur in the testicles of rats with experimentally induced morphine addiction.

Method: Thirty-two Wistar albino rats were divided into four groups: Control (C), Morphine Dependence (M), Morphine Dependence + Morphine Withdrawal (MN), and Morphine Withdrawal (N). Morphine dependence was induced in the M and MN groups. Before decapitation, the rats were evaluated for behavioral changes and scored according to the Geller-Holtzman scale. After decapitation, one testicular tissue sample was placed in Krebs solution for contraction analysis, while the other was fixed in formaldehyde for histopathological examination. Testicular capsule contraction tensions, withdrawal scores, and modified Johnsen scores were assessed.

Results: When the effects of morphine withdrawal were examined in rats injected with morphine, a significant difference was found between the C and MN, C and N, and M and MN groups ($p<0.001$). In isolated organ bath experiments, a significant difference in contraction values before drug application was observed between the C and MN groups ($p<0.05$); however, no significant difference was found in group-time values ($p>0.05$). Histological analyses of testicular tissue sections from the morphine group revealed a decrease in germinal layer thickness and degeneration in the seminiferous tubules. Histopathological examination of testicular tissues showed that the M group exhibited negative effects, while these effects were reversed in the MN group.

Conclusion: At the cellular level, morphine was observed to have negative effects on testicular function, which could be reversed with naloxone. The testicular capsule contraction parameter was not clearly informative. In the morphine addiction model, more detailed insights into testicular contraction functions may be obtained by extending the application period and conducting dose-dependent studies.

Keywords: Isolated organ bath, morphine dependence, morphine withdrawal, naloxone, opioid, testicular contraction

INTRODUCTION

Drug addiction, also known as substance dependence, is a chronically recurrent disorder characterized by the compulsion to seek and use drugs, loss of control over intake, and the emergence of a negative emotional state (e.g., dysphoria, anxiety, irritability) when access is restricted (1).

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Opioids are natural (morphine, codeine) or synthetic (meperidine, fentanyl, sufentanil, alfentanil, remifentanil) compounds found in the central nervous system (CNS) and other tissues. They act by binding to specific receptors and are primarily used for analgesia. Natural alkaloids such as morphine and codeine, along with their semi-synthetic derivatives, are classified as opioids. Pharmacological studies have identified three classes of opioid receptors: mu (μ), delta (δ), and kappa (κ). These receptors are predominantly found in the central nervous system, including the brain and spinal cord, as well as in blood vessels, the heart, lungs, intestines, and even blood mononuclear cells (2). The most commonly used opioids for chronic pain management are μ agonists such as morphine, methadone, and buprenorphine, which may cause addiction with repeated use (3).

Morphine is a μ receptor agonist, and all of its effects are mediated through this receptor. Additionally, morphine exhibits weak affinity for κ and δ receptors, producing various effects through these pathways (4). Although numerous studies have been conducted on morphine, particularly in various regions of the brain tissue where its receptors are densely located, research examining the effects of morphine addiction and withdrawal on other tissues is limited.

Naloxone is a non-selective, short-acting opioid receptor antagonist that has been used clinically for a long time and is widely recognized as a safe drug across a broad dose range. It is used to treat respiratory difficulties caused by opioid overdose. In opioid-dependent individuals, sudden withdrawal from opioids or the administration of μ-opioid receptor antagonists triggers opioid withdrawal syndrome (5). Opioid antagonists such as naloxone and naltrexone reverse and prevent opioid effects by blocking the μ-opioid receptor. The blockade of μ-opioid receptors induces acute withdrawal symptoms in opioid-dependent individuals (6).

The testicular capsule, derived from humans or rodents, is a thin layer of tissue surrounding the testis with contractile properties primarily regulated by catecholamines released from sympathetic nerve endings. The contractile activity of the testicular capsule facilitates the transport of sperm from the seminiferous tubules to the epididymis, and its dysfunction may lead to reduced male fertility (7). Numerous studies have demonstrated the presence of endogenous opioid peptides in various testicular cells. Additionally, these studies have identified and described the presence of three different types of opioid receptors (μ, κ, and δ) in the testis (8).

In light of this information, no study was found in the literature that simultaneously utilized an isolated organ bath and an opioid addiction model to investigate the rat testicular capsule. Understanding the contractile properties of the testicular capsule and examining the changes in these properties in rats with morphine addiction is crucial for evaluating sperm production and the transport of sperm to the epididymis. Impairment of these contractile properties is a significant indicator of male sexual dysfunction. Therefore, this study aimed to observe changes in testicular contractility in rats with experimentally induced morphine dependence.

METHODS

Ethical Approval

The protocols for the animal experiments were approved by the Necmettin Erbakan University Local Animal Experimentation Ethics Committee (approval no. 2021-004) on April 9, 2021.

Experimental Design

Adult male Wistar Albino rats (weighing 300-350 grams, 4 months old) were used in this study. Each group consisted of eight animals, and their distribution was randomized. Throughout the experiment, the animals had ad libitum access to food and water and were maintained under controlled conditions, including a constant temperature of 21±2°C and a 12-hour light/12-hour dark cycle (lights on at 07:00).

Group 1 (Control Group, n=8, C Group): Rats were administered saline solution (SF, 0.9% NaCl isotonic, 10 mg/kg) subcutaneously once daily for seven days. Additionally, on the morning of the seventh day, 3 mL/kg saline was injected intraperitoneally, and the animals were observed for behavioral changes.

Group 2 (Morphine Dependence Group n=8, M Group): Rats were administered morphine (10 mg/kg) subcutaneously once daily for seven days. On the morning of the seventh day, 3 mL/kg saline was injected intraperitoneally, and the rats were observed for behavioral changes.

Group 3 (Morphine Dependence + Morphine Withdrawal Group, n=8, MN Group): Rats were administered morphine (10 mg/kg) subcutaneously once daily for seven days. On the morning of the seventh day, 3 mL/kg naloxone, a μ-opioid antagonist, was injected intraperitoneally to assess addiction.

Group 4 (Morphine Withdrawal Group, n=8, N Group): Rats were administered saline solution (SF, 0.9% NaCl isotonic, 10 mg/kg) subcutaneously once daily for seven days. On the morning of the seventh day, 3 mL/kg naloxone was injected intraperitoneally.

Establishment of Morphine Dependence and Evaluation of Morphine Withdrawal Score

To induce experimental morphine dependence, rats received 10 mg/kg morphine subcutaneously once daily for seven days. To assess the development of morphine dependence, 3 mL/kg naloxone, a μ-opioid antagonist, was administered intraperitoneally. Immediately after naloxone administration, which triggered morphine withdrawal, the animals' behavioral characteristics were monitored and recorded. The observed morphine withdrawal signs were scored accordingly. The presence of significant behavioral signs during morphine withdrawal is considered an indicator of the effectiveness of the applied addiction model. This experimental design has been used in many studies, and it has been confirmed that morphine dependence develops with the same dose applications in conjunction with naloxone administration (9–12).

Geller-Holtzman Scale

The Geller-Holtzman scale is used to determine the morphine withdrawal score. The behaviors of the experimental animals were observed, recorded, and scored according to this scale, which was applied to each animal during the experiment. According to the scale, the behaviors indicating withdrawal are as follows: weight loss (1 point per 1% weight loss), escape attempt (1 point if the number of escapes is between 2-4; 2 points if between 5-9), shaking like a wet dog (2 points if observed 1-2 times; 4 points if observed 3-4 times), defecation (2 points per instance), teeth grinding (2 points per instance), rolling movements (2 points per instance), prominent salivation (7 points), jumping (2 points per jump), pouncing (1 point), self-care behaviors (1 point), ptosis (1 point), swallowing (1 point), and abnormal posture (3 points). All these behaviors were recorded when observed.

Isolated Organ Bath System

The organ bath chambers used in this study have a double-walled structure and contain four chambers. Distilled water heated in a thermocirculator circulates through the outer walls of the organ bath chambers. Thanks to this system, the tissue inside the chamber is maintained at physiological temperature (37°C). The temperature (37°C) is kept constant throughout the experiment. Krebs solution is added to the chambers and 5 mm sections are obtained from the excised tissues. These sections are suspended using a silk thread, with one end attached to the top of the chamber and the other submerged inside. After adjusting the appropriate tension, the tissue section remains in the chamber. The tension frequencies are then monitored using an amplifier, which transfers the electrical signal from the transducers to the recording device.

Following the behavioral procedures, the rats were subjected to cervical dislocation under ketamine (60 mg/kg) and xylazine (6 mg/kg) anaesthesia. After cervical dislocation, the testes were quickly removed and transferred into Krebs solution (composition: NaCl: 119 mM, KCl: 4.7 mM, $MgSO_4$: 1.5 mM, KH_2PO_4: 1.2 mM, $CaCl_2$: 2.5 mM, $NaHCO_3$: 25 mM, Glucose: 11 mM). Once foreign tissues and blood residues were removed, the testicular capsule was suspended in the isolated organ bath. The isolated organ bath was maintained at 37˚C and continuously gassed with a 95% O_2 and 5% CO_2 mixture in Krebs solution. The tension was set to 1 g, and isometric tension changes of the testicular capsule were recorded using a four-channel force transducer. After suspension, the tissues were washed every 15 minutes for one hour to allow the anesthetic agents to wear off. At the end of this one-hour period, 10-3 M adrenaline was administered, and the results were recorded at 15-minute intervals. Contractions were recorded in terms of frequency and tension using the isolated organ bath system (7, 13).

Examination of Histological Parameters

Testicular tissue samples from all groups were fixed in 10% formaldehyde solution for 48 hours. Following routine histological processing, approximately 5 μ-thick sections were obtained. The sections were stained with hematoxylin-eosin and Masson Trichrome stains and subsequently sealed. The stained preparations were examined under a light microscope and photographed. To assess the maturation and quality of the seminiferous tubules, the stained slides were evaluated using the modified Johnsen scoring system at 400x magnification. Ten tubules were analyzed separately for each experimental group, and Johnsen Testicular Biopsy Scoring was applied to each tubule. Scores ranging from 0 to 10 were assigned based on epithelial maturation (Table 1).

Table 1: Johnsen testicular biopsy scoring system

Score 1	No germ cells or Sertoli cells present
Score 2	No germ cells present
Score 3	Only spermatogonia present
Score 4	Only a few spermatocytes present
Score 5	Many spermatocytes present, but no spermatozoa or spermatids
Score 6	Only a few spermatids present
Score 7	Many spermatids present, but no spermatozoa
Score 8	Only a few spermatozoa present
Score 9	Many spermatozoa present, but spermatogenesis is disorganized
Score 10	Complete spermatogenesis with fully developed tubules

Hematoxylin and Eosin Staining Protocol

The sections were placed in an oven at 60˚C overnight for deparaffinization. The sections were then immersed in three different xylene solutions, the first for 20 minutes in the oven and the other two for 10 minutes each. Next, the sections underwent five sequential alcohol dilutions for dehydration, followed by a rinse with distilled water. The sections were then stained with Hematoxylin (BS-001 LOT 022022.021 Harris Hematoxylin, BES LAB) for 10 minutes. After staining, the sections were washed in running water for 10 minutes to remove excess stain, followed by staining with Eosin (Eosin Y Solution, BES LAB) for two minutes. Following the staining process, the sections were passed through a graded alcohol series (70%, 80%, 96%, and two series of absolute alcohol). For clearing, the sections were immersed in three xylene baths for 20 minutes each. Finally, the sections were mounted with Entellan (UN 1866, Merck, Darmstadt, Germany).

Masson Trichrome Staining Protocol

For deparaffinization, the sections were placed in an oven at 60˚C overnight in three different xylene solutions. They were then passed through a graded alcohol series with decreasing concentrations. After rinsing in distilled water, the Masson Trichrome staining set (Masson Trichrome 100-test, Beslab Brand) was applied sequentially. Following staining, the sections were washed under running water and then passed through an increasing alcohol series. After clearing with xylene, the sections were mounted with Entellan.

Statistical Analysis

Statistical analysis for morphine withdrawal scoring was performed using a one-way analysis of variance. Additionally, the mean and standard deviation values for withdrawal scoring data were tabulated. A mixed-effects model was used to assess changes in stress values between groups, over time, and within groups. Analyses were conducted using SAS University Edition 9.4 software, with $p<0.05$ considered statistically significant. For Johnsen scoring, mixed-effects models were used to compare numerical variables. Tukey corrected least square means were compared post hoc. These analyses were performed using R 4.3.2 (R Core Team, 2024), with $p<0.05$ considered statistically significant.

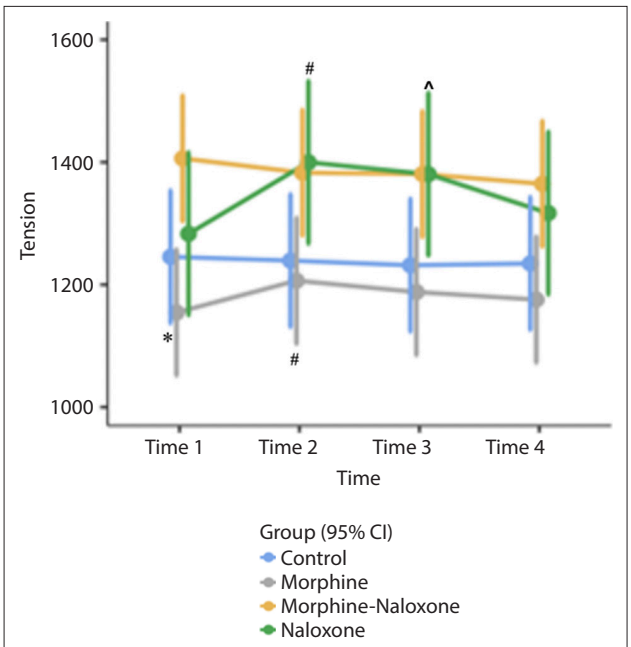

Figure 1. Tension values of testicular capsules before and after induction with adrenaline.

*A significant difference was found between the C and MN groups at Time 1 ($p<0.05$); #A significant difference was found between the M and N groups between Time 1 and Time 2 ($p<0.001$); ^A significant difference was found in the N group between Time 1 and Time 3 ($p<0.05$).

RESULTS

Withdrawal Syndrome Parameters

Withdrawal results based on Gellert-Holtzman withdrawal scale scoring (Table 1), along with mean values and standard deviations, are presented in Table 2. While the withdrawal score was 19.55±3.16 in the control group, it was 13.9±4.93 in the morphine group. The morphine-naloxone group had a withdrawal score of 50.2±6.54, while the naloxone group had a score of 7.16±1.86. A significant difference was found between the groups ($p<0.001$). According to post hoc analyses, a significant difference was found between the C and MN, C and N, and M and MN groups ($p<0.001$).

Table 2: Mean values (±standard deviation) of withdrawal syndrome findings

	Control group	Morphine group	Morphine + Naloxone group	Naloxone group
Weight	368.88±90.71	364.8±43.18	308.9±30.69	344.33±37.54
Weight loss (g)	11.77±5.35	5.2±3.15	10.7±3.68	8±2.38
Escape attempts	7.88±5.03	7.9±4.45	5.5±2.17	3.33±0.94
Wet dog shakes	0±0	0±0	1.7±1.15	0.16±0.37
Defecation	1.5±1.5	0.9±0.99	5.7±1.49	1.5±0.5
Teeth chattering	0.66±1	0.3±0.48	4.9±2.02	1.16±0.68
Rolling movement	0±0	0.1±0.31	0.3±0.48	0±0
Profuse salivation	0±0	0±0	0.7±0.82	0±0
Jumping	2.55±1.23	2.5±1.84	0.2±0.63	0.5±0.5
Self-care	4.1±1.76	2±1.33	3.8±2.39	0±0
Ptosis	2.11±1.53	0.8±1.135	5.7±2	0±0
Swallowing	0.77±1.3	0±0	0.6±1.07	0±0
Abnormal posture	0.33±0.5	0.9±0.87	2.5±0.84	0±0
Erection, ejaculation, or genital grooming	0±0	0±0	0±0	0±0
Withdrawal score	19.55±3.16	13.9±4.93#	50.2±6.54*	7.16±1.86*

*Significantly different from the control group, p<0.001; #Significantly different from the MN group, p<0.001.

Isolated Organ Bath Parameters

The mean tension value of the testicular capsule was recorded at 15 minutes before adrenaline administration as Time 1, 15 minutes after as Time 2, 30 minutes after as Time 3, and 45 minutes after as Time 4. The isolated organ bath findings are presented in Figure 1. Analysis using the mixed-effects model indicated that both group effect (p=0.026) and time effect (p=0.036) were statistically significant. However, the group*time interaction effect was not significant. A significant difference was found between the C and MN groups when comparing values 15 minutes before adrenaline administration (p= 0.036).

Additionally, a significant difference was observed in the M group and N group when comparing values 15 minutes after adrenaline administration to those 15 minutes before administration (p=0.048; p<0.001). Furthermore, in the N group, a significant difference was detected when comparing the tension value 30 minutes after adrenaline administration with the tension value recorded 15 minutes before adrenaline administration (p<0.05, p=0.004) (Fig. 1).

Histological Analysis of Testicular Tissues

Histological sections of testicular tissues were stained with hematoxylin and eosin (H&E) and subsequently evaluated. Microscopic examination of testicular sections from the C group revealed seminiferous tubules with a normal structure, a basal membrane lined with spermatogonia, scattered Sertoli cells, and an orderly arrangement of germinal cells (spermatids and spermatozoa) of the maturing spermatogenic series. The interstitial connective tissue and Leydig cells within the interstitial area exhibited normal morphology (Fig. 2a). In contrast, testicular sections from the M group displayed abnormal morphological features, including degeneration and atrophy of seminiferous tubules as well as irregularities in germ cells (Fig. 2b). In the N group, most seminiferous tubules retained their original structure, and the general histological appearance of the testes was nearly normal (Fig. 2c). In the MN group, mild loss and irregularities in germinal epithelial cells were observed in some tubules. Partial degenerative changes were also noted in Leydig cells within the interstitial spaces (Fig. 2d).

Table 3: Group tubule interaction values of Johnsen scores

Contrast	Estimate	SE	df	t. ratio	p
C - M	3.986	0.187	24.000	21.284	<**0.001**
C - N	0.743	0.187	24.000	3.967	0.003
C - MN	1.429	0.187	24.000	7.629	<**0.001**
M - N	-3.243	0.187	24.000	-17.317	<**0.001**
M - MN	-2.557	0.187	24.000	-13.655	<**0.001**
N - MN	0.686	0.187	24.000	3.662	0.006

SE: Standard error; df: Degrees of freedom.

Johnsen Scores Analysis

Additionally, the mean Johnsen scores for 10 randomly selected seminiferous tubules from each group were evaluated based on the criteria presented in Table 1. Statistically significant differences were found among the groups ($p<0.05$). The highest scores were observed in the C group, while the lowest scores were recorded in the M group (Fig. 3, Table 3).

Masson's Trichrome Analysis

Masson's trichrome staining was used to visualize collagen fibers and indicate fibrosis. In the C group, collagen fibers were observed in the walls of blood vessels within the interstitial area (Fig. 4a). In the M group, an increase in collagen fibers was detected in both the walls of blood vessels and the basal membranes, compared to the control group (Fig. 4b). The N group demonstrated collagen fibers in vessel walls similar to the control group (Fig. 4c). Lastly, in the MN group, a reduction in collagen fiber density in vessel walls and basal membranes was noted compared to the morphine group (Fig. 4d).

DISCUSSION

In this study, the effects of morphine dependence and withdrawal on testicular tissue in male rats were investigated. For this purpose, behavioral symptoms, contraction patterns of the testicular capsule, and testicular tissue were evaluated histopathologically in morphine-dependent and withdrawal-induced rats. An addiction model was created using morphine, an opioid commonly used in the treatment of acute and chronic pain, at specific doses and durations.

As a result of this study, morphine withdrawal syndrome was observed in morphine-injected rats, and its presence was found to be statistically significant. When the rats were examined behaviorally, wet dog shaking and salivation were observed only in the MN group. In addition to these behaviors, prolonged rolling movements were observed in both the morphine and MN groups.

Opioid use disorder causes a variety of effects, with most studies focusing on its impact on CNS. However, research has also explored its effects on the reproductive system and testicular functions. Studies have shown that plasma testosterone reduction, loss of libido, decreased ejaculation volume, and reduced sperm motility are associated with heroin and methadone use (14, 15). One of the key findings from multiple studies on the effects of opioid use on the male reproductive system is a significant decline in the effectiveness of sex hormones and spermatogenesis (16–19).

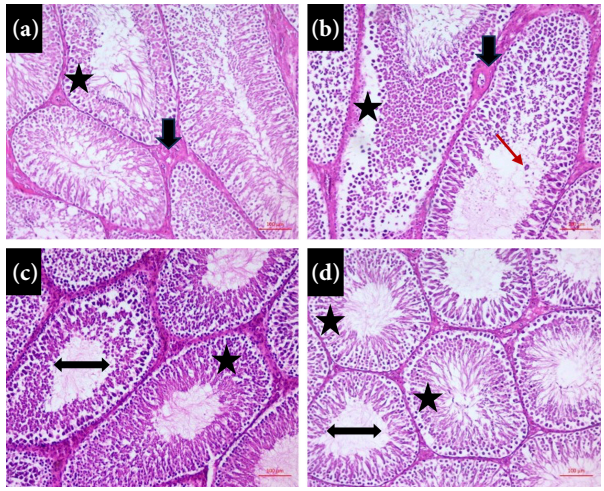

Figure 2. **(a)** Control group (C): Preserved tubules, spermatogenic cells (star), normal interstitial area (thick short arrow). **(b)** Morphine group (M): Degeneration of spermatogenic cells and shedding of the germinal layer (star), interstitial area changes (thick short arrow), and residual body in the lumen (red thin arrow). **(c)** Naloxone group (N): Preserved tubules and spermatogenic cells (star), dense spermatozoa in the tubule lumen (double-sided arrow). **(d)** Morphine + Naloxone group (MN): Improvements in tubules and spermatogenic cells (star), spermatozoa in the tubule lumen (double-sided arrow). Hematoxylin and eosin (H&E) staining, 10x.

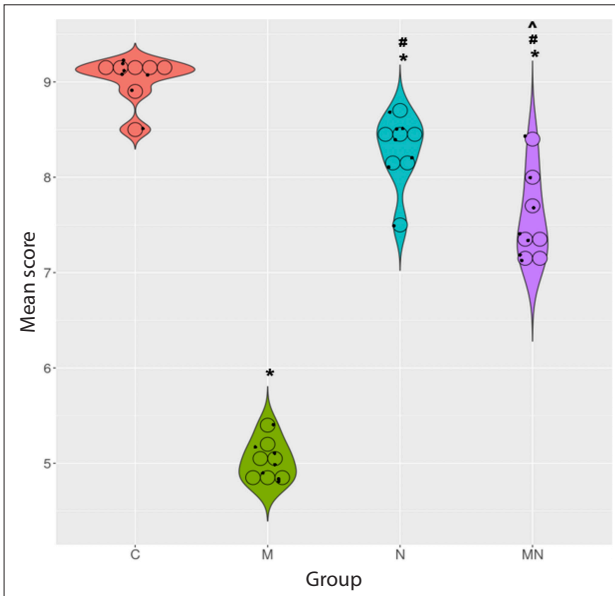

Figure 3. Comparison of mean Johnson scores between groups.

*A significant difference was found between the C and M, N, and MN groups ($p<0.05$); #A significant difference was found between the M and N, MN groups ($p<0.001$); ^A significant difference was found between the N and MN groups ($p<0.01$).

Opioid receptors widely distributed throughout the body, including in various parts of the reproductive system. Numerous studies have demonstrated the presence of endogenous opioids in different testicular cells. Additionally, research has confirmed the presence of three types of opioid receptors in rat testes. These receptors have been identified in Sertoli cells, spermatozoa, and Leydig cells (20, 21).

The efficiency of the male reproductive system depends on the proper functioning of a complex system involving multiple organs, the appropriate release of gonadotropes, and the cooperation of androgens and local hormones. The proper functioning of these systems is essential for spermatogenesis. It is well known that morphine abuse causes damage to both central and peripheral nervous system mechanisms, leading to a significant reduction in sex hormone levels, spermatogenesis, and a decreased adult sperm count (22).

Endogenous opioid peptides are present in various tissues, including the male reproductive system. Therefore, these opioids are believed to play an important role in reproductive functions. They reduce testosterone production by inhibiting gonadotropin-releasing hormone (GnRH) release, which in turn suppresses testosterone secretion. However, no opioid has been found to have a significant effect on follicle-stimulating hormone (FSH) levels (23, 24). A prospective experimental study investigated the histopathological changes associated with tramadol administration, a μ-opioid receptor agonist. Unlike previous studies, this research found degeneration in the seminiferous tubules (25). A researcher who extensively studied the male reproductive system, particularly the storage of morphine flow and detoxification by methadone, examined the seminal vesicles, testes, luteinizing hormone (LH), FSH, and testosterone levels. The study revealed a significant difference in LH and testosterone levels between the control group and the morphine-only group. In the morphine + methadone group, the only significant change compared to the control group was a decrease in testosterone levels (26). Morphine affects almost all stages of spermatogenesis. In a study investigating the impact of morphine addiction on spermatogenesis in rats, a significant decrease was observed in the spermatogonia, spermatocyte, spermatid, and spermatozoa stages, all key cell types involved in sperm development (22). Additionally, morphine administration has been shown to reduce testicular weight by affecting the number of Sertoli cells and germinal cells in the testis, as well as by impairing sperm production. This negative effect is believed to result from the production of free radicals and a reduced antioxidant capacity in the testis, ultimately leading to the death of testicular cells and sperm (27).

Since opioid receptors are also present in testicular tissue, morphine may inhibit spermatogenesis by directly affecting these receptors in testicular tissue. A separate study demonstrated that thymoquinone treatment led to an improvement in germinal layer thickness, and its protective effects were significantly reduced in morphine-treated groups compared to other groups. Additionally, it was observed that seminiferous tubule cells differentiated more rapidly in morphine-treated groups than in the other groups (28). In the present study, a decrease in germinal epithelial layer thickness and degeneration of spermatogenic cells were observed in the seminiferous tubules.

Naloxone regulates the release and inhibition of various hormones associated with testicular function. It is widely used in animal models to induce physical withdrawal symptoms in opioid addiction studies and to interact with the endogenous opioid system. Studies have shown that naloxone administration during neonatal testicular development can increase the number of Sertoli cells per testis, the total length of seminiferous tubules, daily sperm production per testis, and daily sperm count (29).

In this study, separation and irregularity in germ cells, as well as degenerative changes in seminiferous tubules, were observed in the testicular tissues of rats in the M group. The Johnsen score was also significantly reduced compared to the control group. However, the testicular structure in the N group was similar to that of the control group. In the MN group, naloxone application was found to mitigate the damage caused by morphine and increase the Johnsen score compared to the M group. The histopathological findings of this study suggest that naloxone may help reduce the degeneration caused by morphine in testicular tissue.

Many studies investigating smooth muscle contraction in animal models of morphine addiction and withdrawal have primarily focused on the small intestine and vascular system. Among the systems most affected by opioid addiction, the gastrointestinal system experiences a variety of side effects. The most common side effects include abdominal pain, vomiting, and constipation. These gastrointestinal symptoms place an additional burden on patients with

chronic pain, negatively impacting their quality of life and, in some cases, altering the effectiveness of opioid addiction treatment. In general, morphine, fentanyl, and their derivatives are opioid agonists widely used for perioperative pain management. However, studies have shown that constipation is the most common and distressing side effect associated with opioid agonists used for chronic pain management (30, 31).

Research focusing on the digestive system has found that morphine increases small intestinal motility, leading to constipation, a common symptom in morphine addiction. Although we expected blood pressure values to decrease in the morphine group compared to the C group in our study, no significant difference was found between the two groups. However, a non-significant decrease was observed in the morphine group compared to the control group. Our hypothesis was that the morphine group would reduce the contraction tension of the testicular capsule. Due to certain limitations of this study, no significant changes may have been observed in the testicular capsule contraction parameter. The main limitations include the morphine dose and the duration of administration. These factors can be adjusted and improved in future studies.

CONCLUSION

Opioid addiction has become a global health problem. Morphine addiction and withdrawal syndrome have numerous negative effects on human health. While there are studies investigating the effects of morphine and other opioids on various smooth muscle cells, such as those in the aorta, uterus, bladder, trachea, and small intestine, there is no study in the literature that explores behavioral changes and testicular contraction parameters in rats with morphine addiction. In this study, an addiction model was established using morphine, an opioid commonly used in the treatment of acute and chronic pain, administered at specific doses and durations. This research examined behavioral responses, testicular contraction parameters, and histological changes in rats with morphine addiction and withdrawal. Results showed that morphine dependence and withdrawal syndrome successfully induced in rats and were found to be statistically significant. While we expected the morphine group to exhibit a reduction in testicular capsule contractile tension, the observed decrease in the morphine group compared to the control group was not significant. The Johnsen score of the morphine group was found to be significantly lower than that of the other groups. However, the MN and N groups showed significant improvement in histological findings. Although both morphine dependence and withdrawal syndrome were successfully induced with the current study design, more distinct results regarding testicular tissue contraction stress-frequency and animal behavior in the isolated organ bath could be obtained by increasing the application time and dose.

Contribution Categories		Author Initials
Category 1	Concept/Design	O.Y., Z.I.S.G.
	Data acquisition	O.Y., Z.I.S.G.
	Data analysis/Interpretation	O.Y., Z.I.S.G.
Category 2	Drafting manuscript	O.Y., Z.I.S.G.
	Critical revision of manuscript	O.Y., Z.I.S.G., R.O.K, H.S., B.G.
Category 3	Final approval and accountability	O.Y., Z.I.S.G., R.O.K, H.S., B.G.

Ethical Approval: The Necmettin Erbakan University, Application and Research Center of Experimental Medicine Ethics Committee granted approval for this study (date: 09.04.2021, number: 2021-004).

Conflict of Interest: The authors declare that they have no conflict of interest.

Informed Consent: Informed consent was obtained from all participants.

Use of AI for Writing Assistance: Not declared.

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Peer-review: Externally peer-reviewed.

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