Effects of Opium Dependency on Testicular Tissue in A Rat Model: An Experimental Study

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Purpose: This study is aimed to evaluate the effects of opium dependency on testicular tissue in a rat model.

Methods: Thirty-two Wistar male rats (aged 30 days and weighing 200-250 grams) were randomized into two groups. Group A, consisting of 16 rats, received dissolved oral opium tablets in drinking water for 45 days, whereas group B (control group) consisted of 16 rats that received opium-free water. After 45 days vertical and horizontal diameters of testis, number of seminiferous tubules, mean seminiferous tubule diameter, number of germ cells, height of germinal epithelium, percentage of degenerating Leydig and germ cells and glutathione density of testicular tissue (µmol/g of tissue) were compared between study groups.

Results: Morphological evaluation of testicular tissue revealed a significantly higher percentage of degenerating Leydig and germ cells in the treated group compared to control group. (10.08 ± 0.351 vs. 1.83 ± 0.88, 4.50 ± 0.769 vs. 0.607 ± 0.118, respectively) (P-value<0.001 for each) Interestingly, vertical and horizontal diameter of testis, the average number of germ cells, height of germinal epithelium and number of seminiferous tubules, were significantly higher in the treated group compared to control group. Seminiferous tubule diameter and glutathione density of testicular tissue were not statistically significantly different between the groups.

Conclusion: Applying a rat model, we noted that opium has a substantial effect on testicular structure and function. A significantly higher proportion of Leydig and germ cells were degenerated in treated rats despite an increase in the average number of seminiferous tubules and germ cells. These findings support the hypothesis that opium consumption adversely affects male fertility.

Keywords: animal models; infertility; opium; testis

INTRODUCTION

Opioids has been used widely for their analgesic effects. Furthermore, opioid abuse is common in some regions in the world and have been postulated to be associated with infertility in men.[1] The opioid system, including endogenous opioid peptides and opioid receptors, modifies secretion of gonadotropin-releasing hormone (GnRH), and subsequently alters the serum levels of follicle-stimulating hormone (FSH) and luteinizing hormone (LH). It has been shown that activation of opioid receptors is associated with decreased serum LH levels, whereas, opioid antagonists, including naloxone, increase serum LH levels.[2,3] Opioids may modulate gonadal functions via binding to opioid receptors in the hypothalamus, the pituitary gland, and the testes(4,5). Several studies have demonstrated acute and chronic effects of endogenous and exogenous opioids in regulating sex hormone secretion, including testosterone and estradiol. Opioid dependency has been shown to decrease serum levels of testosterone, LH, and FSH, and consequently may be associated with decreased libido, erectile dysfunction, and infertility in men.[6,7] Decreased sperm motility after morphine administration has also been observed in some studies, a finding that underscores potential role of opioid system in regulating sperm motility.[4,8] Besides endocrine effects, opioids might also directly damage testicular and ovarian tissues. Some studies with contradicting findings have evaluated role of opioid agonists and antagonists in oxidative stress in different organs.[9-11] Despite extensive evaluations addressing endocrine effects of opioids, studies evaluating the histomorphological and oxidative-related effects of opium on testicular tissue are insufficient. We conducted this experimental study to assess the impact of opium on testicular tissue in a rat model.

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Table 1. Comparison of histopathological parameters between study groups.

	Opium-treated rats, Group A (N=16)	Control rats, Group B (N=15)	*P*-value
Number of germ cells	109.50 ± 4.63	82.47 ± 4.96	< 0.001
Seminiferous tubule diameter	229.38 ± 20.63	216.20 ± 16.79	0.062
Height of germinal epithelium	76.63 ± 3.83	73.27 ± 1.79	< 0.001
Number of seminiferous tubules	35.50 ± 4.90	27.60 ± 3.20	< 0.001
Vertical diameter of testis (mm)	12.468 ± 0.670	10.383 ± 1.671	< 0.001
Horizontal diameter of testis (mm)	8.796 ± 0.922	7.258 ± 0.535	< 0.001

Data are shown in mean ± SD.

MATERIALS AND METHODS

Animals

Thirty-two Wistar male rats (aged 30 days and weighting 200-250 grams) were randomly assigned into two groups. Group A (treated group, n=16) consisted of 16 rats that received dissolved oral opium tablets in drinking water for 45 days. Group B (control group, n=16) received opium-free water. Both groups were kept in a 12/12 hours dark/light cycle, air-conditioned environment with controlled temperature and humidity, and were treated with food and tap water ad libitum throughout the study. International standards for the care of laboratory animals were followed and the protocol of this experimental study was approved by institutional ethical committee (Approval number: 90-03-114-15256).

Opium dependency

In rats of group A, addiction was induced by treating with dissolved oral opium tablets in drinking water for 45 days. Each tablet contained 100 mg opium (10 mg morphine). At the beginning of the study, opium tablets were added to drinking water in group A to a concentration of 1 mg/mL. Opium concentration increased by 1 mg/mL every 48 hours to the maximum concentration of 4 mg/mL, which was continued to the end of the study. Rats in group B were maintained in similar condition and received opium-free water. The changes in the daily amount of water intake was also recorded in all rats. To assess opium dependency, two rats from each group were randomly selected and received 2 mg/kg intra-peritoneal naloxone. Opium dependency was confirmed 20 minutes after injection of naloxone by occurrence of withdrawal symptoms, including writhing, ptosis, diarrhea, jumping, teeth chattering, head and wet-dog shaking, and paw tremor in rats of group A.

After 45 days, the rats were killed with CO_2 asphyxiation and bilateral orchiectomy was performed through a midline scrotal incision. Immediately after the operation, one testis was placed in Bouin's solution for histological evaluation and another testis was stored at –80°C for biochemical assays.

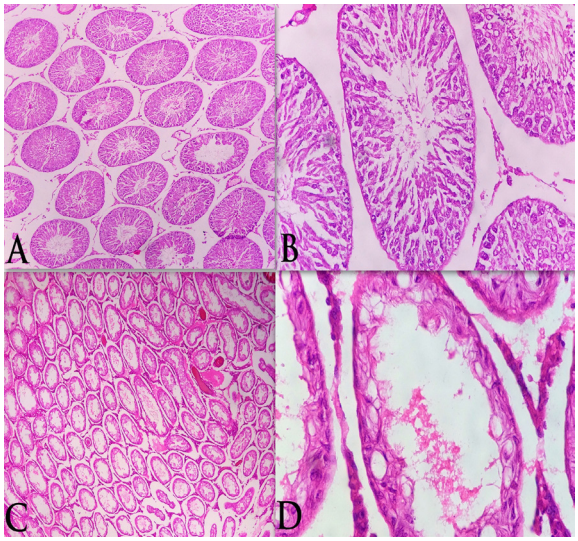

Figure 1. Histopathological evaluations of testicular damage in opium-dependent rats. Histopathological evaluation of testicular tissue revealed significant cell degeneration in the opium treated group **(C, D)** compared to control group **(A, B)**.

Histopathological studies

Histopathological assessment of testicular tissues was performed by a blinded pathologist. After preparation of paraffin blocks, tissue sections with 5 µm thickness from the mid-portion of testicular tissue was provided and stained with hematoxylin and eosin. Examination with an optical microscope under magnification of 10-100 times with standard technique was carried out. Sertoli cell only syndrome was diagnosed in one rat in control group which was excluded from the study. Testicular length and width were measured by ocular micrometer microscope. Tubules were counted in an area of 1500 µm × 1500 µm centered in the middle of the field. The seminiferous tubule diameter, number of germ cells, height of germinal epithelium and the percentage of degenerating germ cells were recorded in 20 seminiferous tubules. Percentage of degenerating Leydig cells was also assessed in 20 inter-tubular spaces.

Biochemical studies

Frozen testicular tissues were homogenized, centrifuged and prepared for measuring glutathione concentration as an index of oxidative damage after morphine administration. Measurement of glutathione concentration of testicular tissue was performed applying BIOXYTECH® GSH-400 kit (OXIS International, Inc., Portland, OR, USA). The results were recorded as µmol per gram (µmol/g) of tissue.

Statistical Analysis

Data were analyzed using SPSS (SPSS, Inc., Chicago, Illinois) version 15. We used the Chi-square or Fisher's exact test to compare qualitative data. Student's t test, and Mann-Whitney U test were applied to compare quantitative data. *P*-value < 0.05 was considered as statistically significant.

RESULTS

In the course of the study, weight of rats in both groups and the amount of daily water intake were recorded. Average weight of rats and their mean water intake were comparable between the study groups. Furthermore, no significant change was observed in the amount of water

intake in group A, as the concentration of morphine in drinking water increased.

Morphological evaluation of testicular tissue revealed a significantly higher percentage of degenerating Leydig and germ cells in the treated group compared to control group (10.08 ± 0.351 vs. 1.83 ± 0.88, 4.50 ± 0.769 vs. 0.607 ± 0.118, respectively) (*P*-value < 0.001 for each). The proportion of degenerating cells was noted to be more than 5 times in rats of group A compared to group B (**Figure 1**). Despite degeneration of Leydig and germ cells, we found that vertical and horizontal diameters of testis, the average number of germ cells, height of germinal epithelium and number of seminiferous tubules, were significantly higher in group A. Moreover, seminiferous tubule diameter was comparable between rats of the study groups. **Table 1** compares various histopathological parameters between study groups. However, we did not find a statistically significant difference in Glutathione density of testicular tissues between two groups of the study (16 ± 1.5 vs. 15 ± 1.4 µmol/g in group A and B, respectively; *P*-value=0.818).

DISCUSSION

Endocrine effects of opioids have been extensively reviewed in the literature, however, our study was one of the few studies to assess the effects of opium on histomorphological parameters of testis. Our results revealed that opium consumption is associated with significant detrimental effects on testicular histomorphology and produces degenerative changes in testicular tissue.

Opioid peptides are postulated to play an important role in regulation of testicular function. Animal studies have shown that opiate receptors exist in Sertoli cells and opioids are capable of modifying the response of Sertoli cells to FSH.[12-14] Endogenous opioid peptides also bind to opioid receptors on gonadotropic cells, in the pituitary gland, and inhibit GnRH release. Therefore, endogenous opioid peptides are involved in controlling reproductive function at different stages.[15]

In a study evaluating the effects of morphine sulfate injection on rat reproductive system, investigators showed decreased serum LH and testosterone levels, as well as reduction in spermatogenic cells. Although testicular weight was not affected by morphine administration, prostate and seminal vesicle weights decreased significantly. Spermatid development was also affected in morphine treated rats with reduced counts of both early and late spermatids. Furthermore, they noted decreased tubular diameter and Sertoli cell counts as a consequence of morphine administration.[16]. In a similar study, Abdellatief et al. reported that chronic consumption of tramadol in rats, leads to decrease in serum LH, FSH and testosterone levels. They also noted that rats treated with tramadol have more destruction of seminiferous tubules, separation of tubular basement membrane, decrease in seminiferous tubules diameter and germinal epithelial height.[17] Additionally, El Sawy et al. noted that administration of tramadol for one month could lead to suppression of spermatogenesis and exfoliation of germ cells inside the lumina of the tubules.[18] In the present study we noted an increase in the average number of germ cells, although this increase was concurrent with significant increases in number of degenerated cells. Increased number of germ cells in our study, although statistically significant, does not seem to be of clinical implication and does not preclude toxic effects of opioids on testicular tissue. Simultaneous presence of hyperplasia and degenerative processes have been reported in several studies addressing histopathological changes in various tissues.[19,20] These findings highlight the hypothesis that observed increases in number of germ cells might be more attributable to tissue responses against opioid toxic effects, rather than benign histopathological changes.

Some studies have also assessed impacts of opioid antagonists on testicular tissue. Naloxone, as an opioid antagonist, has been reported that can increase release of gonadotropin-releasing hormone (GnRH) and block inhibitory effects of stress on testosterone production in rats. It is also reported that naloxone treated rats have more spermatozoids and sertoli cells, as well as increased tubular length, sexual cords, sperm production and testicular weight.[21-23]

Although studies concerning the effects of opioid agonists on testicular tissue are insufficient, several reports have investigated effects of these substances on the hypothalamic pituitary gonadal axis, both in animals and humans.[15,16,24,25] Yilmaz et al. have reported that chronic consumption of opioids does not affect seminiferous tubules and Leydig cells, but it can suppress releasing GnRH, LH and testosterone hormone, without altering serum FSH level. Later, Padmanabham et al. confirmed FSH can be released without GnRH stimulation.[26]

Besides the alterations in endocrine regulation, opium consumption may result in oxidative damages to testicular tissues. Opium induced oxidative damage has not been evaluated in the literature. However, studies have shown detrimental effects of cocaine and cigarette smoke on testicular tissue. Li et al. evaluated cocaine induced oxidative damage in testicular tissue in a rat model. They showed that cocaine impacts on spermatogenesis, reduces testicular level of glutathione, an antioxidant agent, and induces apoptosis in rat testes.[27] Similarly, cigarette smoke affected testicular antioxidant enzyme levels and impaired spermatogenesis in rats.[28,29] In a similar paper, Kushwaha et al. reported that nicotine abuse augments testicular toxicity in diabetic rats.[30] In the present study, we assessed effects of opium on testicular glutathione density in opium dependent rats. No significant difference was noted in glutathione density in opium dependent rats compared to control group. However, it should be considered that lack of difference in glutathione density between study groups may be pertinent to the limited power of the study.

Applying a rat model, we noted that opium has a substantial effect on testicular structure and function. A significantly higher proportion of Leydig and germ cells were degenerated in treated rats despite an increase in the average number of seminiferous tubules and germ cells. These findings support the hypothesis that opium consumption adversely affects male fertility. However, our study is associated with certain limitations including limited sample size and lack of re-review of pathology slides and further studies are required to confirm our findings.

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CONFLICT OF INTERESTS

Authors declare that there are no competing interests.

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