# The relation of opium addiction and reproductive toxicity in male rats: a histological and hormonal study

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## Abstract

**Background and objective:** Consuming of opium is a new phenomenon that could be significantly observed in some regions of Iraqi Kurdistan region, especially in districts near to Iran borders. Long term consumption of opioids affects body homeostasis. This study is designed to determine the effects of opium on reproductive histological and hormonal parameters in addition to the study of qualitative sperm abnormalities in experimental rats.
**Methods:** The rats of the experiment were divided into three groups: Control group and the two experimental groups which were treated with two different concentrations of opium (25 and 50 mg/kg/day) for 7 days. After the decided period, the rats were dissected. Serum testosterone and sperm quality was determined and histological sections were prepared from the testis.
**Results**: Testosterone significantly reduced in opium treated rats in both low and high doses. The histological sections of testis showed testicular degeneration in the seminiferous tubules, while higher dose showed loss of normal architecture of seminiferous tubules, in addition to present of giant cell in lumen of tubules. Several types of sperm abnormalities were observed, but the head-neck connection abnormality was the dominant.
**Conclusion:** This finding suggests that opium addiction can cause significant decrease in the male sexual hormone secretion and it also leads to the alteration in the sperms and testis structure. This may lead to sexual suppression and infertility which needs further investigations.
**Keywords:** Opium-Sperm- Testosterone-Histology- Testis

## Introduction

Opium abuse is a major problem for every society including Iraq. More than 180 million people have tried illegal drugs once and there are 13.5 million opium addicts in the world [1]. However, several researches has been focused on heroine in investigations about drugs addiction because of its spreading in western countries, but in our region, opium is mostly used because it is very common in Iran which considered as the main source of drugs for our country. It has been demonstrated that a great changes in the sexual activity happened in addicted subjects. The effects of drug abuse on sexual functions and sex hormones are one of the major scopes of investigations throughout the world. Since heroin and cocaine consumption is the most popular drug of abuse in western countries the majority of studies have focused on heroin and cocaine addicted subjects [2,3,4]. Since 1803, when morphine was isolated from the opium poppy (papaver somniferous) by Serturner [5], abuse of opium and related derivatives has continued to exponentially increase around the world [6]. Morphine, an opiate alkaloid, is most frequently processed chemically to produce heroin, a significant contributor to the state of the current drug trade [7]. Substance abuse has been shown to affect the hypothalamic pituitary- gonadal axis and its relationship with endocrine system [8].

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Morphine consumption results in increased hormone levels including adrenalin, noradrenalin, corticosterone, and glucagon [9–12], which, in turn, affect the metabolism in different ways. Opioid exposure can cause alterations in both the physiology and structure of the kidneys. The related clinical implications are significant [13]. Little is known about the histological and reproductive actions of opium in experimental animals. Therefore, our investigation is designed to evaluate the effects of different concentrations of opium on some reproductive histological and some hormonal parameters, besides to the morphological study of sperm in male rats.

## Methods

### Drug Preparation

The extract of Opium was received from the directorate of narcotics control in Erbil province-Iraq. The stock solution of Opium was prepared by dissolving 2.5 gms of opium extract in 50ml of 35% diluted ethanol producing a concentration of (50mg/ml). Amount of 1ml of the prepared solution was injected intraperitoneally to each 1kg of rat body weight (50mg/kg). While, further dilution was made for preparation of 25mg/kg rat b.w. [5].

### Experimental Design

The rats of the experiment were divided into three groups:

control group (6 rats): The rats of this group were injected with 35% ethanol solution (1ml/kg).

opium 1st group (6 rats): The rats of this group were received with intraperitoneal injection of 25mg/kg/day opium drug for a week. All injections were done in the morning.

opium 2nd group (6 rats) : The rats of this group were treated with intraperitoneal injection of 50mg/kg/day opium drug for a week. All injections were done in the morning. After seven days, the rats of all groups were anesthetized, the blood samples were collected from direct heart puncture and the rats then dissected for other evaluations and tests.

### Sperm preparation

The sperms were prepared from epididymis and vas deference. After killing the animals, the epididymis and vas deference were removed from the testes and transferred in to small petridish containing normal saline. By using a sharp scissor the epididymis and vas deference were cut into several parts, the sperms were released into the saline solution. The sperm suspension were smeared and dried, fixed with fixative (ethanol was used as a fixative), finally stained with 1% Eosin stained for 5 min. the slide washed by distilled water and were left to dry [14].

### Histological Preparation of Testis

Testis samples were removed from the anesthetized animals. All samples were fixed in bouin's fluid and processed for light microscopy by embedding in paraffin after dehydration and clearing. Six micrometers thick sections were stained by haematoxylin and eosin [15].

### Testosterone Assay

Serum concentration of total testosterone were measured for all samples using an automated quantitative system (Mini Vidas; bioMerieux, Lyon,France). All samples were analyzed using the enzyme linked fluorescent assay technique, an enzyme immunoassay sandwich method with a final fluorescent detection.

## Results

### Histological study

The histological sections of testis belong to rats treated with 25 mg /kg opium showed alteration in the seminiferous tubules represented by testicular degeneration, edema and infiltration of mononuclear inflammatory cells between seminiferous tubules, Figure 2. While, Histological section of rats testis treated with opium showed alterations represented by loss of normal architecture of seminiferous tubules, some of these tubules showed testicular degeneration in addition to present of giant cell in lumen of tubules and some revealed loss of semen whereby no spermatozoa were present, Figure 3. Whereas Figure 1

shows the histological sections for control rats. shows the histological sections for control rats.

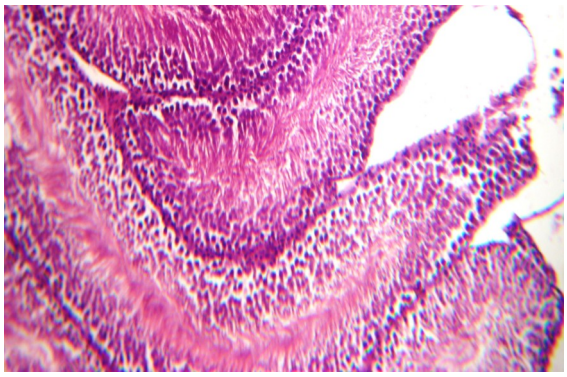

**Figure 1:** Histological section of control rats (400X)

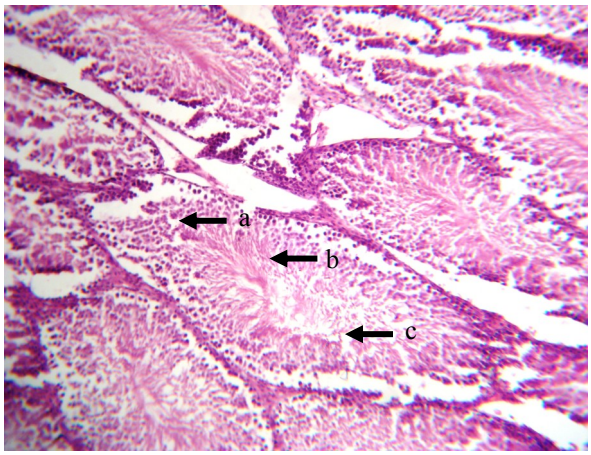


**Figure 2:** histological section of rat testis treated with 25 mg/kg opium showed loss the normal structure of seminiferous tubules, (**a**) infiltration of mononuclear inflammatory cells, (**b**) edema and (**c**) testicular degeneration. 100X

## Testosterone level

The result of current study reported that a dramatically reduction in testosterone level was occurred in opium treated rats in both low and high doses. The level of testosterone in control group was 3.522 ±0.57 ng/ml, while the level was dropped to 0.422±0.13 ng/ml as it is illustrated in Figure 4. The testosterone level was near to zero in the high dose of opium.

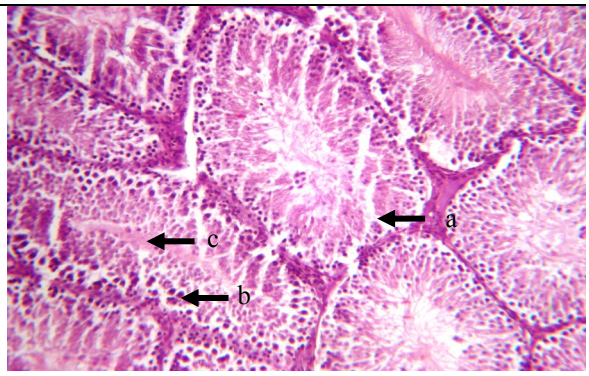


**Figure 3:** Histological section of rats testis treated with 50 mg/kg opium showed lesions represented by loss normal architecture of seminiferous tubules, some of these tubules showed (a) testicular degeneration (b)in addition to present of giant cell in lumen of tubules and some revealed loss of semen (c) no spermatozoa were present.(400X)

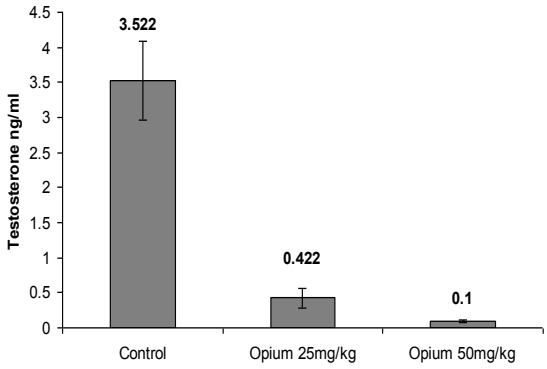


**Figure 4:** Testosterone levels ng/ml in control and opium treated rats.

## Sperm Quality study

Several types of abnormalities in sperm were observed in rats treated with opium, especially with high dose. The dominant type of abnormality was head –neck connection abnormalities, Figure 6 while normal sperms were found in control group, Figure 5.The other types of abnormalities include: tail abnormalities like the presence of loop, Figure 7 or drops, Figure 8, also defective hooks and heads, Figure 9 and 10 were clearly observed in most of the fields.

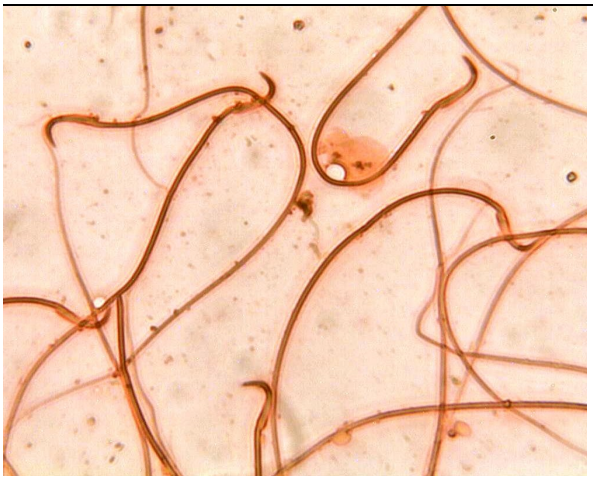

Figure 5: normal sperms from control group rats

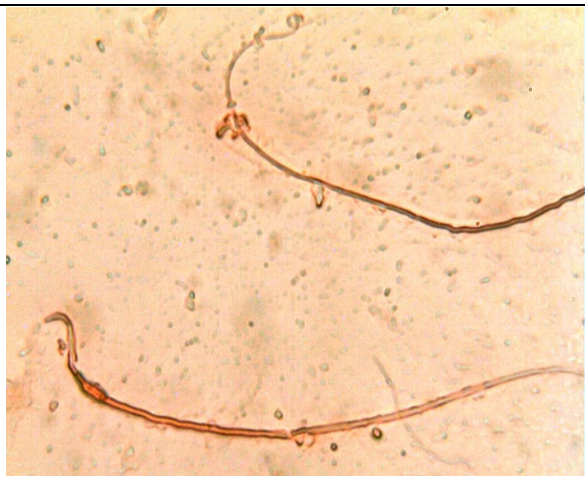

Figure 8: Dropped tail abnormal sperm

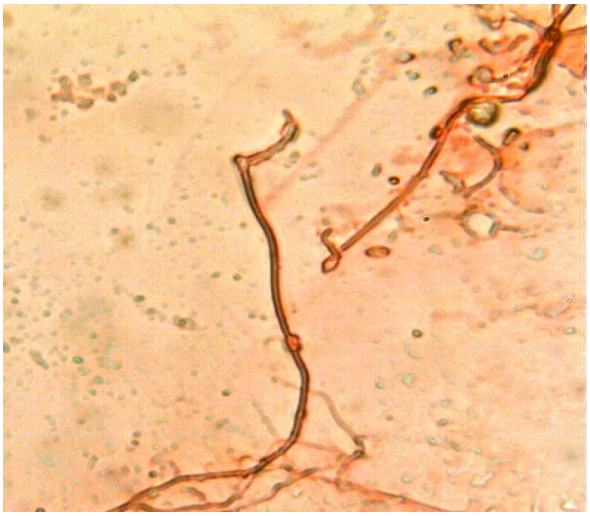

Figure 6: Head-neck abnormal sperm

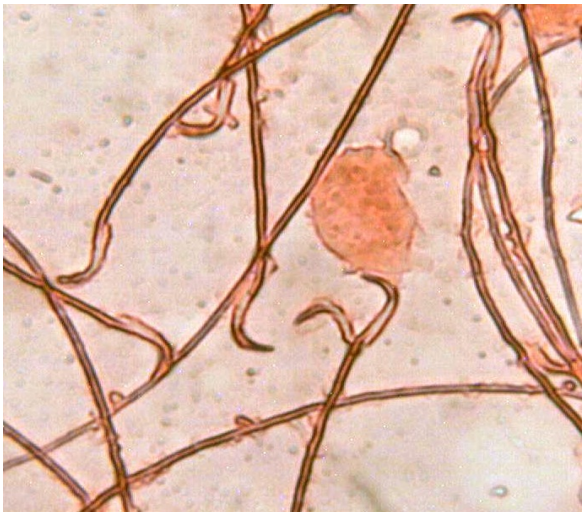

Figure 9: Double head abnormal sperm

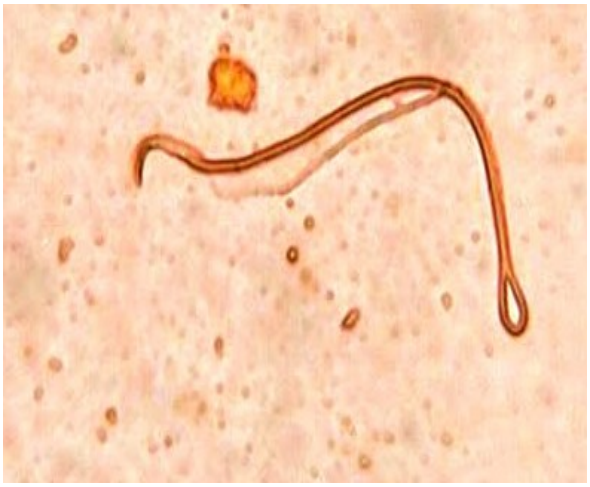

Figure 7: Looped tail abnormal sperm

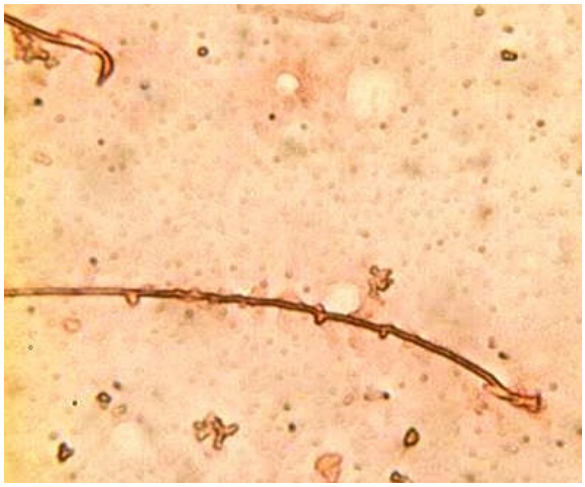

Figure 10: Defective hook sperm

## Discussion

Apparently, despite opium consumption considered to be a declining trend in most of the societies, recent data have shown notable opium usage in some developing countries, such as Iran. Several reports have shown opium to be the predominant form of substance used in Iran. In Iran 1 in 17 persons (i.e., 5.8%) is a regular drug user and 20% of Iranians, aged 15 to 60, are involved in drug use [16-18]. Our finding reveals that chronic use of opium in male rats cause toxicity in the testis through altering the architecture of the seminiferous tubules and producing inflammations. However of few researches about the toxicity of opium on testis histology, similar findings have been documented by [19] who suggests acute pulmonary edema due to a direct toxic effect of opium. Also [20] studied the action of opium on kidney and concluded that renal damage may progress to terminal renal insufficiency. The current results also reported that opium addiction may cause great reduction of sexual activity through declining the level of testosterone which was near to zero in high dose of drug. This result was supported by Hassan and Muhamad [21] who suggests that the chronic use of opium can cause significant decrease in the functions of hypophysiol gonadal secretion which may lead to sexual suppression and infertility which needs further investigations. The action on sperm quality was complementary to the hormonal action. Several types of sperm abnormalities were detected in addicted rats. This may be directly through its action on gonads or via hypothalamic-hypophysiol-gonadal axis [22]. The gonadotropines (LH, FSH) are pulstily released from hypothalamus and acts via hypotalamo-hypophysiogonadal axis which stimulate gonadal endocrine function and gametogenesis in males. This activity leads to proper spermatogenesis and male sexual responses [22]. Suppression of this axis will lead to reduction of sperm count, semen quality, impairment of erection and finally infertility [23].

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