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Research Article

## Effects of oxycodone hydrochloride on reproductive functions in male wistar rats

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### Abstract

Drug iatrogenicity is a constantly evolving phenomenon, affecting several therapeutic classes. The aim of the present study was to investigate the effects of prolonged administration of oxycodone hydrochloride, a central analgesic, on reproductive functions in male rats. **Materials and methods:** three groups of 5 rats each were created; distilled water 0.5 ml/100g, oxycodone hydrochloride 5 and 10 mg/kg. The different products were administered orally daily for 30 days. The variables studied were: animal behaviour, prostate, testicular and epididymal weights, spermogram parameters and hormonal biomarkers (FSH, LH and testosterone levels).

**Results:** behaviorally and morphologically, the results obtained show that oxycodone hydrochloride at doses of 5 and 10 mg/kg causes stiffness of the animals' tails, priapism and atrophy of the prostate, testicles and epididymis, of which intensity was dose-dependent. On spermogram parameters, oxycodone hydrochloride caused a significant decrease in sperm motility ( $p<0.001$ ) and in the number of normal spermatozoa ( $p<0.05$ ). Administration of oxycodone hydrochloride to rats significantly decreased testosterone levels ( $p<0.05$ ) and an increase in LH levels.

**Conclusion:** Oxycodone hydrochloride, administered to animals at doses of 5 and 10 mg/kg, causes morphological disturbances of the gonads and related structures, spermogram parameters and gonadal hormones.

**Keywords:** oxycodone hydrochloride, spermatogenic and hormonal parameters, gonads, rat

## INTRODUCTION

Opioid consumption has increased considerably over the last three decades, particularly in developing countries<sup>1</sup>. Their main indication is the management of chronic pain and palliative treatment, which partly explains the considerable increase in their use. The rise in the abuse of opioid medications has become a public health challenge globally. However, chronic opioid use can have a number of adverse effects on organ systems, including the reproductive system, significantly affecting patients quality of life<sup>2,3</sup>. Abs and al (2000)<sup>4</sup> report that opioids impair male fertility via different mechanisms. At high doses, the concentrations of testosterone decline with associated hypogonadism<sup>4,5</sup>. Opioids have been shown to decrease reproductive parameters in male rats<sup>6</sup>. They increase the production of reactive oxygen species (ROS) and can adversely affect spermatogenesis, leading to sexual dysfunction or infertility<sup>3</sup>. Opioid-related adverse effects are receptor-mediated and therefore inseparable from treatment<sup>7</sup>. Several studies have been conducted with rodent models to investigate the adverse effects of gestational opioid consumption on the neurological development of offspring, but there is little data regarding the effects of Exposure to opioids (including oxycodone) on reproductive functions in adult rats<sup>8</sup>. Opioids

have a pharmacological action on the hypothalamic-pituitary or gonadal axis. Some changes have been observed, such as an increase in prolactinemia, a decrease in plasma cortisol and in testosterone. Oxycodone, a  $\mu$ -opioid receptor agonist, was synthesized in 1916 and introduced into clinical use in Germany in 1917<sup>9</sup>. It has been studied extensively as a therapeutic analgesic for acute and chronic neuropathic pain as an alternative to morphine<sup>6</sup>. Oxycodone emerged as a drug with widespread abuse. The therapeutic effect is mainly analgesic, anxiolytic, antitussive and sedative<sup>3,6</sup>. In this study, we investigate the effects of repeated administration of oxycodone hydrochloride in male rats on reproductive.

## MATERIALS AND METHODS

### Animals and treatment

Adult male rats of the Wistar strain weighing between 100 and 300 grams were used. They came from the animal house of the Faculty of Health Sciences in Brazzaville. They were reared in polypropylene cages under standard conditions with free access to food and drinking water. All experiments were conducted in compliance with EU Directive 2010/6106/EU on

the protection of laboratory animals<sup>10</sup>. Animals were divided into 3 groups of 5 animals each and treated as follows:

- the first group received distilled water at 5 ml/kg and served as a control batch;
- the second group was treated with oxycodone hydrochloride at a dose of 5 mg/kg ;
- the third group was treated with oxycodone hydrochloride at a dose of 10 mg/kg.

The products were administered orally for 30 days.

The doses of oxycodone hydrochloride used in this study corresponded to the therapeutic doses used in human clinical practice<sup>2,3</sup>.

#### **Sacrifice of animals and collection of samples**

Twenty-four hours after the last treatment, all the animals were sacrificed by inhalation of ether. After incision of the carotid artery, the blood was collected in dry tubes, centrifuged and the serum obtained was used for hormone assays (FSH, LH and testosterone). After dissection, the prostate, testicles, epididymides, spermiducts and seminal vesicles were removed for morphological analysis. After sampling, the organs were immediately weighed and the weight index, corresponding to the ratio between the body weight and the mass of the organ, was calculated. The epididymis and spermiducte were excised, dilacerated and then gently crushed in 1 ml of physiological water. These organs were then removed to obtain a fixed volume of sperm suspension, which was used to determine the sperm parameters of the Wistar rat.

#### **Semen analysis**

Sperm quality was assessed by spermogram and spermocytogram. The variables analysed were: sperm pH,

leucocyte count, sperm motility, sperm count, vitality and morphology. Sperm motility was assessed by direct examination of a drop (20 µl) under a light microscope at X40 magnification. To determine sperm concentration, the sperm suspension was diluted 1:20 with 10% formalin and counted using a Mallassez cell on optical microscope. A sperm suspension in 2% eosin was used to assess sperm vitality. Live and dead sperm were counted under the light microscope. Sperm morphology was assessed after staining a sperm smear with May Grunwald Giemsa. The smear was read under a light microscope. This assessment was made according to the Kruger classification (1986)<sup>11</sup>.

#### **Hormones levels**

We used the direct ELISA (Enzyme Link Immuno Sorbent Assay) with the Cypress® kit to measure FSH, LH and testosterone, following the manufacturer's instructions.

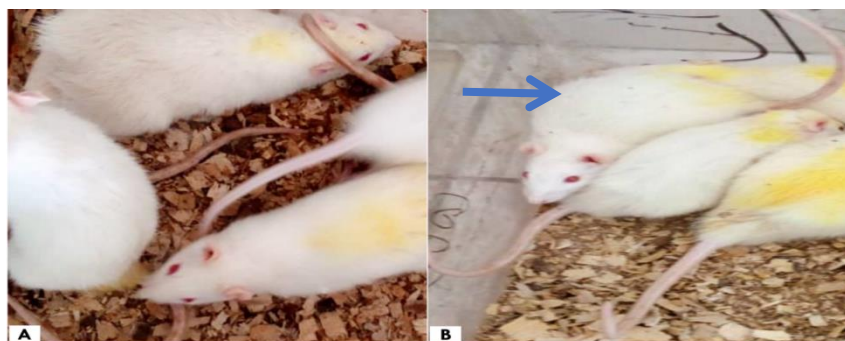
#### **Statistical analysis**

Data were entered into Excel 2019 (Microsoft). Results were expressed as mean ± standard error (SEM). For each variable, the groups of treated animals were compared with the control group. Comparisons of non-evolutionary parameters were made using *Student's t-test* with Statistical Package for Social Science (SPSS), version 20 and Microsoft Excel 2019. The significance threshold corresponds to a risk of error of  $p < 0.05$ .

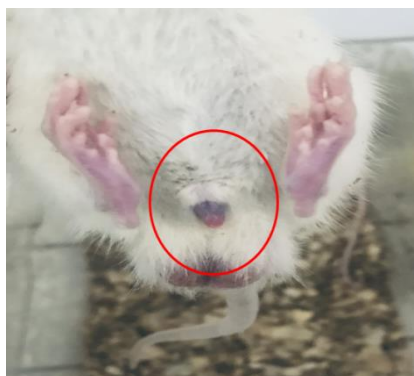
## **RESULTS**

#### **Behavioural aspects**

Animals treated with oxycodone hydrochloride at doses of 5 and 10 mg/kg showed stiffness of the tail and priapism during the period of exposure to the drug. These manifestations are illustrated in figures 1 and 2.



**Figure 1:** Illustration of tail stiffness (A and B) in Wistar rats during exposure to oxycodone hydrochloride.



**Figure 2:** illustration of priapism in Wistar rats during exposure to oxycodone hydrochloride.



**Figure 3:** Photography of the testes of control rats (A and B) and those treated with oxycodone hydrochloride at doses of 5 and 10 mg/kg respectively (C, D, E and F).

### Effects on testicular morphology

Animals treated with oxycodone hydrochloride showed testicular atrophy, the effect of which was dose-dependent. Figure 3 illustrates the morphological differences between the testes of control rats and those treated with oxycodone hydrochloride.

### Organ weight

Prolonged administration of oxycodone hydrochloride for thirty days results in a significant reduction in the weight of the prostate, testes and epididymides. Organ weight indices are shown in Table I.

**Table I:** organ weight index

| Organs           | ED 5 ml/kg | Oxy 5 mg/kg             | Oxy 10 mg/kg |
|------------------|------------|-------------------------|--------------|
| Prostate         | 1,64±0,33  | 0,53±0,16**             | 0,36±0,13**  |
| Left testicle    | 1,73±0,63  | 0,56±0,39**             | 0,69±0,47**  |
| Right testicle   | 1,76±0,63  | 0,58±0,39**             | 0,93±0,51*   |
| Left epididymis  | 0,64±0,26  | 0,51±0,23 <sup>NS</sup> | 0,34±0,13*   |
| Right epididymis | 0,58±0,24  | 0,50±0,20 <sup>NS</sup> | 0,33±0,13*   |

Results are expressed as mean ± standard error. (\*):  $p<0.05$ ; (\*\*):  $p<0.001$ ; NS (not significant) by student's *t*-test. DE: distilled water; Oxy: oxycodone hydrochloride.

### Sperm parameters

The effects of oxycodone hydrochloride on sperm parameters are shown in Table II. Analysis of these results shows that

administration of oxycodone hydrochloride caused a significant decrease in sperm motility ( $p<0.001$ ), number of spermatozoa ( $p<0.05$ ) and percentage of normal sperm morphology ( $p<0.0001$ ).

**Table II** Effects of oxycodone hydrochloride on sperm parameters

| Parameters                                   | DE 5 ml/kg | Oxy 5 mg/kg             | Oxy 10 mg/kg            |
|----------------------------------------------|------------|-------------------------|-------------------------|
| pH                                           | 6,75±0,28  | 6,12±0,25 <sup>NS</sup> | 6,37±0,25 <sup>NS</sup> |
| Total sperm motility (%)                     | 85,0±4,08  | 18,75±37,5***           | 17,5±3,5***             |
| Vitality (%)                                 | 95,25±0,5  | 83,75±7,5 <sup>NS</sup> | 73,75±7,5*              |
| Number of spermatozoa (×10 <sup>6</sup> /ml) | 20,75±2,5  | 25±5,03*                | 31,25±2,5*              |
| Normal morphology (%)                        | 72,50±5,0  | 48,75±4,78*             | 46,25±11,08*            |

Results are expressed as mean ± standard error. (\*):  $p<0.05$ ; (\*\*):  $p<0.001$ ; (\*\*\*):  $p<0.0001$ ; NS (not significant) by Student's *t*-test. DE: distilled water; Oxy: oxycodone hydrochloride.

### Hormones levels

Oxycodone hydrochloride administration to rats significantly decreased testosterone levels only at 10 mg/kg ( $p<0.05$ ). The

effects of oxycodone hydrochloride on hormonal parameters are shown in Table III.

**Table III:** Effects of oxycodone hydrochloride on hormonal parameters

| Parameters   | Distilled water 5 ml/kg | Oxy 5 mg/kg             | Oxy 10 mg/kg            |
|--------------|-------------------------|-------------------------|-------------------------|
| FSH          | 0,32±0,08               | 0,39±0,16 <sup>NS</sup> | 0,32±0,12 <sup>NS</sup> |
| LH           | 0,47 ±0,34              | 0,82±0,41**             | 0,80±0,37**             |
| Testosterone | 1,48± 0,35              | 0,41±0,13**             | 0,29± 0,10***           |

Results are expressed as mean ± standard error. (\*):  $p<0.05$ ; (\*\*):  $p<0.001$ ; (\*\*\*):  $p<0.0001$ ; NS (not significant) by student's *t*-test. DE: distilled water; OxyN: oxycodone hydrochloride.

## DISCUSSION

The use of opioids is known to reduce levels of male sex hormones, and it is thought that this drop in hormone levels is responsible for the reduced fertility of men who take opiates<sup>12</sup>. Oxycodone belongs to the same family as codeine, morphine and tramadol. The aim of the present study was to evaluate the effects of oral administration of oxycodone hydrochloride at doses of 5 and 10 mg/kg for four weeks on reproductive functions in male rats.

Administration of oxycodone hydrochloride in male rats at the doses studied produced behavioural changes manifested by tail stiffness and priapism. In the investigation of male infertility, morphological evaluation of the testicles is of great importance. Indeed, it supports the hypothesis of a testicular mechanism of infertility<sup>13</sup>. In this study, testicular atrophy was observed in the groups of animals treated with oxycodone hydrochloride. Animals treated with oxycodone hydrochloride showed a significant decrease in testicular weight compared with control animals. The same observation was made for the prostate and epididymides.

Sperm quality is recognised as the best predictor of natural fertility<sup>14</sup>. It is assessed through sperm density, vitality, mobility and morphology. It is accepted that these parameters are directly correlated with fertility<sup>15</sup>. In the present study, treatment with oxycodone hydrochloride caused a significant decrease in the mobility, vitality and number of normal spermatozoa. Grizard (1997)<sup>16</sup> reported that changes in pH can indicate a defect in seminal vesicle secretion (decrease in pH) or an insufficiency of prostatic secretion (increase in pH). Morphine has also been reported to increase aromatase expression in the brain and impair testicular function<sup>17,18,19</sup>. Other work has demonstrated that exogenous opioids cause DNA fragmentation and adversely affect sperm quality. These effects are associated with all opioids<sup>20</sup>. In this study, a decrease in pH was observed in the batches of animals treated with oxycodone hydrochloride. However, this decrease was not significant. This decrease in pH could be associated with a deficit in seminal fluid secretion and could explain the apparent increase in sperm density. Thus, the observed increase in sperm count would correspond rather to a concentration of spermatozoa due to hyposecretion of the seminal vesicles.

Testicular functions are regulated by the neuroendocrine axis, which comprises hypothalamic GnRH neurons and pituitary cells that secrete FSH and LH in response to GnRH. Testosterone is produced by Leydig cells in response to LH. In this study, a significant increase in LH levels was observed in animals treated with oxycodone hydrochloride at doses of 5 and 10 mg/kg. Oxycodone hydrochloride did not affect FSH levels in rats. However, there was a significant decrease in testosterone levels at 10 mg/kg. Consumption of opiates (including oxycodone) is known to reduce levels of male sex hormones<sup>4,21</sup>. This depletion of testosterone should be compared with the testicular atrophy observed. Indeed, it has been reported that a moderate decrease in gonadotropin secretion causes testicular atrophy, as well as a decrease in sperm production<sup>22</sup>. The suggested mechanism of this testosterone depletion would involve an increase in prolactin and an inhibition of gonado-pituitary tropin secretion<sup>21</sup>. Furthermore, Leydig and germ cells are the sites of endogenous opioid production. Their receptors are present throughout the testis<sup>23</sup>. The secretion of androgen transport proteins intratesticularly is also inhibited by opioids<sup>21</sup>.

Other authors have also reported a decrease in FSH, LH and testosterone levels caused by opioids<sup>24</sup>. Similar observations were made in rodents treated with heroin which significantly reduced sperm viability and testosterone levels<sup>25</sup>.

## CONCLUSION

Administration of oxycodone hydrochloride at doses of 5 and 10 mg/kg to male Wistar rats reduces testicular, prostate and epididymal weights and induces asthenospermia associated with necroteratozoospermia. This exposure also leads to an increase in LH levels and a depletion of testosterone. Further in-depth studies would help to confirm these disturbances and elucidate the molecular mechanisms involved.

## Conflicts of interest

All the authors declare that they have no conflicts of interest in relation to this work. This study is part of an end-of-study Master's dissertation and has not benefited from any funding from organisations related to the subject.

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## Authors contributions

- ✓ Participated in research design: LM Miguel, AE Mboundou Malonga, AL Nkanga Samba.
- ✓ Conducted experiments: LM Miguel, AE Mboundou Malonga, C Lekana, H Akassa.
- ✓ Contributed new reagents or analytic tools: D Mbemba Bahamboula, CR Dobhat-Doukakini.
- ✓ Performed data analysis: LM Miguel, D Moukassa, AA Abena
- ✓ Wrote or contributed to the writing of the manuscript: LM Miguel, D Moukassa, AA Abena.

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