



## Effect of Tramadol on Sperm Profile of Male Albino Rats

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### Authors' contributions

*This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.*

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### ABSTRACT

**Background and Aim:** Tramadol is a potent analgesic effective in the treatment of mild to severe pains. However, the use of the drug can pose a threat to other organs and systems. Therefore, this study evaluated the effect of graded doses of tramadol on sperm profile of male albino rats.

**Materials and Methods:** Eighteen male rats were divided into three groups (A, B and C) using completely randomized design (CRD) with six rats in each group. Rats in group A served as the control group and were given just food and water while groups B and C were given tramadol at 50 and 100 mg/kg body weight (BW) respectively, daily for the period of 65 days. The treatment was administered via oral gavage and at the end of the treatments, the rats were sacrificed. Immediately after sacrifice, a puncture was made in the epididymis with a sterile pin and examined for semen pH. The epididymes were processed for epididymal sperm motility, viability, count and sperm head abnormality.

**Results:** There was no significant difference in the weight of testes and semen pH. Sperm viability, sperm motility, sperm count and weight of epididymes significantly reduced ( $p < 0.05$ ) in tramadol treated animals when compared with the control. Results also indicated statistically significant ( $p < 0.05$ ) increase in sperm head abnormalities in rats treated with tramadol when compared with the control.

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**Conclusion:** The results obtained from this study reveal that tramadol has negative effects on weight of epididymes, sperm count, sperm viability, sperm motility and sperm head abnormalities in male albino rat as mammalian models in a dose dependent manner.

*Keywords: Tramadol; sperm profile; sperm quality; sperm quantity and sperm head abnormalities.*

## 1. INTRODUCTION

Tramadol is a synthetic 4-phenyl-piperidine analogue of codeine. It is a centrally acting analgesic used in the treatment of different levels of pain ranging from moderate to severe, acute or chronic [1,2]. The efficacy of tramadol was considered to be one tenth to one-sixth that of morphine [3,4]. Furthermore, tramadol has been considered to be an effective form of treatment for premature ejaculation at a low and safe therapeutic dose and provided a new option for managing mild to severe premature ejaculation [5].

However, the adverse effects of tramadol are generally similar to those of opioids, although they are not as severe as those of opioids and include respiratory depression, dysphoria, constipation, and central nervous system depression [6–8]. El-Gaafarawi [9] observed changes in the biochemical profiles of tramadol users in the form of increased liver and kidney functions and decreased sex hormones. An increasingly alarming rate of tramadol abuse has been reported the last four years [10].

Generally, opioids are used as analgesic drugs without considering the several side effects already known. One of the side effects that is rarely considered is hypogonadism [11,12]. In recent times, it has been observed that intrathecal and oral opioids are capable of suppressing testosterone secretion throughout their period of administration [13–16]. Opioids, both endogenous and exogenous, modulate gonadal function primarily by acting on opioid receptors in the hypothalamus [17], inducing the decreased release or disruption of the normal pulsatility of gonadotropin releasing hormone secretion. This results in a reduction in the release of the luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the pituitary gland and of testosterone or estradiol (E2) from the gonads. Opioids can also have direct effects on the pituitary gland and the testes [18].

Hence, the study was aimed at examining the effect of tramadol treatments on sperm profile of male albino rats.

## 2. MATERIALS AND METHODS

### 2.1 Location and Duration of Study

This study was carried out in the Department of Genetics and Biotechnology, University of Calabar, Calabar, Cross Rivers State of Nigeria. The study lasted 65 days.

### 2.2 Experimental Animals

Eighteen healthy and sexually mature male albino rats of 12 weeks old of average body weight of 176.8g were used in this study. The rats were obtained from the Experimental Animal Unit of Department of Genetics and Biotechnology, University of Calabar, Calabar. The rats were housed in conventional wire mesh cages under standard laboratory conditions of 12 h light and dark cycle. They were allowed free access to water and pellet feed throughout the period of the experiment. Generally, the study was conducted in accordance with the recommendation from the declarations of Helsinki on guiding principles in care and use of animals and the local ethical committee (Approval number: CRS/MH/CGS&EH/Vol.2/14).

### 2.3 Experimental Design and Procedure

The rats were divided into three groups (A, B and C) using completely randomized design with six rats in each group using simple random sampling technique. Rats in group A served as the control group and were given just food and water while groups B and C were given tramadol at 50 and 100 mg/kgBW respectively daily for the period of 65 days. The treatment was administered via oral gavage and at the end of the treatment the rats were sacrificed. The weights of testes and epididymes were obtained using Scout Pro SPU 601 electronic weighing balance. The epididymes were then processed for epididymal sperm motility, viability, count and sperm head abnormality and semen pH. Immediately after dissection, a puncture was made in the epididymis with a sterile pin. The semen smeared on the pin was rubbed on a pH paper of range 4.0-10.0. The colour change corresponds to the pH and was read from the paper. Two

drops of sperm suspension was put on a microscope slide and cover slip was placed. The number of progressively motile cells was divided by the total number of spermatozoa counted under x40 lenses and expressed as a percentage [19].

## 2.4 Sperm Viability

The sperm viability test was determined using "Eosin-Nigrosin one-step staining technique" [19]. A portion of the sperm suspension was mixed with equal volume of Eosin-Nigrosin stain and five (5) air-dried smears were prepared on glass slides for each sample. The slides were examined for percentage viability. Normal live sperm cells excluded the stain and appeared whitish, whereas dead sperm cells took up stain and appeared pinkish. Percentage viability was calculated based on the number of live sperm cells out of the total number of sperm cells observed.

## 2.5 Sperm Count

The epididymal sperm samples were obtained by macerating known weights of caudal epididymes in physiological saline in the ratio of 1:10 weight by volume. After vigorous pipetting to release the sperm cells. The suspension was filtered using an 80 µm stainless mesh. Epididymal sperm count was obtained by cytometry using the improved Neubauer cytometer (Model: BR723014) and was expressed as million/mL of suspension [20].

## 2.6 Sperm Head Abnormality Test

A portion of the sperm suspension was mixed with 1% eosin Y solution (10:1) for 30 min and air-dried smears were prepared on glass slides for the sperm head abnormality test. The slides were examined for percentage sperm head abnormalities in every 200 sperm cells observed on each slide and five air-dried smears were prepared on glass slides for each sample. The

percentage of sperm head abnormality was calculated according to Ekaluo et al. [21].

## 2.7 Statistical Analysis

Data from weight of testes and epididymes, epididymal semen pH, motility, viability, count and sperm head abnormality were subjected to one-way Analyses of Variance (ANOVA) test at 5% level of significance while differences in means were separated using Least Significant Difference (LSD) test.

## 3. RESULTS

### 3.1 Weight of Testes and Epididymes

There was no significant effect of tramadol on the weight of testes of the treated rats when compared with the control (Table 1). Meanwhile, a significant decrease in the weight of epididymes was observed in tramadol treated animals when compared with the control as shown in Table 1.

### 3.2 Sperm Profile

Results on the sperm profile of animals in the treatment and control groups are presented in Table 2. There was no significant difference in the semen pH in the treatment groups when compared with the control. However, a significant ( $p < 0.05$ ) dose effect was observed in other sperm parameters studied. Sperm motility significantly reduced ( $p < 0.05$ ) in tramadol treated rats from 75.85% in the control to 39.04% in group B treated with 50mg/kgBW of tramadol. Also, sperm count and sperm viability also decreased significantly ( $p < 0.05$ ) in rats treated with tramadol in a dose dependent manner with sperm count declining from  $7.84 \times 10^6$ /ml in the control to  $5.95 \times 10^6$ /ml in group C (100mg/kgBW of tramadol). Sperm viability also declined from 81.79% in the control to 61.23% in group C (Table 2).

**Table 1. Effect of Tramadol treatments on weight of testes and epididymes of albino rats**

Parameter	A (Control)	B (50 mg/kgBW)	C (100 mg/kgBW)
Testes weight (g)	1.32 <sup>a</sup> ±0.03	1.22 <sup>a</sup> ±0.04	1.23 <sup>a</sup> ±0.05
Epididymes Weight (g)	0.43 <sup>a</sup> ±0.03	0.34 <sup>b</sup> ± 0.025	0.34 <sup>b</sup> ± 0.025

Mean values with different superscript along the same row are significantly different from each other at 5% level of significance ( $P < 0.05$ ).

**Table 2. Effect of Tramadol treatments on sperm parameters of male albino rats**

Parameter	A (Control)	B (50 mg/kgBW)	C(100 mg/kgBW)
Semen pH	7.35 <sup>a</sup> ± 0.025	7.22 <sup>a</sup> ±0.035	7.23 <sup>a</sup> ±0.025
Sperm motility (%)	75.85 <sup>a</sup> ± 1.80	39.04 <sup>b</sup> ± 4.70	44.32 <sup>b</sup> ± 2.37
Sperm viability (%)	81.79 <sup>a</sup> ± 2.20	62.72 <sup>b</sup> ± 1.50	61.23 <sup>b</sup> ±2.90
SHA (%)	7.64 <sup>a</sup> ± 0.55	10.79 <sup>b</sup> ±0.35	12.58 <sup>c</sup> ±0.57
Sperm count (x10 <sup>6</sup> /ml)	7.84 <sup>a</sup> ±0.55	6.34 <sup>b</sup> ±0.35	5.95 <sup>b</sup> ±0.20

Mean values with different superscripts along the same row are significantly different from each other at 5% level of significance ( $P<0.05$ ). SHA = Sperm head abnormality

On the other hand, sperm head abnormalities significantly increased in group of rats treated with the graded doses of tramadol in a dose dependent manner. The percentage of abnormal sperm heads increased from 7.64% in the control group to 12.58% in group C treated with the high dose of tramadol as shown in Table 2.

#### 4. DISCUSSION

The results obtained in this study revealed that tramadol treatment significantly affected the weight of epididymis and other sperm parameters studied which agrees with the findings of El-Gawet [22] while no significant impact was observed in semen pH and weight of testes. Some studies had earlier revealed that epididymes shrink and degenerate as a result of long term use of tramadol [23].

The reduction in the sperm profile in tramadol treated animals could be as a result of its effect on spermatogenic processes and pathways in the animals. This assertion is supported by Ezzat and El-Gohary [24] and Ikpeme et al. [25] who reported that disruptions in fertility in male mammals has direct correlation with disruptions in spermatogenesis. Therefore, this implies that tramadol might have impaired spermatogenesis with a concomitant decrease in sperm count and weight of epididymes. The reduction in sperm count corroborates the decrease in weight of epididymes observed in tramadol treated rats.

Moreover, degenerative changes in the histology of the testis of rats have been reported to cause a decline in the testosterone levels and consequently distorts spermatogenesis [26-27]. This might be the underlying cause of the significant reduction in the sperm count and weight of epididymis observed in tramadol treated animals.

Results obtained also revealed a significant ( $p<0.05$ ) increased in sperm head abnormalities

in tramadol treated animals which is indicative of induced mutation of genes on the sperm cells during the spermatogenic processes in line with the findings of Glover and Assinder [28], Uno et al. [29] and Ikpeme et al. [25].

#### 5. CONCLUSION

The findings of the study provide substantial evidence that tramadol has an adverse effect on sperm profile and reproductive organs of male albino rats in terms of weight of epididymes, sperm count, sperm viability, sperm motility as well as sperm head abnormalities.

#### ETHICAL APPROVAL

The study was conducted in accordance with the recommendation from the declarations of Helsinki on guiding principles in care and use of animals and the local ethical committee (Approval number: CRS/MH/CGS&EH/Vol.2/14).

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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