

**HISTOPATHOLOGICAL EFFECTS OF TRAMADOL TREATMENTS ON THE TESTES OF MALE ALBINO RATS**

**ABSTRACT**

Tramadol is effective in the treatment of moderate to severe pains. However, abuse of the drug can have negative impact on other organs and physiological processes. Hence, this study was aimed at determining the histopathological effect of tramadol treatments on the testes of male albino rats. Eighteen male rats were divided into three groups: control, T<sub>1</sub> and T<sub>2</sub> using completely randomized design (CRD) with six rats in each group. Rats in group A were the control group and were given just food and water and groups T<sub>1</sub> and T<sub>2</sub> were given tramadol treatments at 50 and 100 mg/kgBW respectively, daily. The treatments were administered via oral gavage daily for 65 days and at the end of the treatment the rats were sacrificed using chloroform anaesthesia. There was no significant difference in the weight of testes. Sperm count and weight of epididymes significantly reduced ( $p < 0.05$ ) in tramadol treated animals when compared with the control. Histological examination reveal that tramadol treated rats had lumen with fewer spermatids with slight necrosis, atrophy and inflammation in T<sub>1</sub> treated rats while severe inflammation and haemorrhage around the Leydig cells were observed in T<sub>2</sub> treated animals indicating a dose dependent testicular toxicity and degeneration when compared with the control group. The results obtained from this study indicate that tramadol treatments has deleterious effects on weight epididymes (from 0.425g in the control group to 343g for T<sub>1</sub> and T<sub>2</sub> animals, respectively), sperm count, and testicular integrity in male albino rat as mammalian models in a dose dependent manner.

**Keywords:** Tramadol, opioid, histopathology, male gonads, sperm count.

**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<sup>5</sup>.

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

40            Generally, opioids are used as analgesic drugs without considering the several side  
41 effects already known. One of the side effects that is rarely considered is hypogonadism  
42 [11,12]. In recent times, it has been observed that intrathecal and oral opioids are capable of  
43 suppressing testosterone secretion throughout their period of administration [13–16]. Opioids,  
44 both endogenous and exogenous, modulate gonadal function primarily by acting on opioid  
45 receptors in the hypothalamus [17], inducing the decreased release or disruption of the  
46 normal pulsatility of gonadotropin releasing hormone secretion. This results in a reduction in  
47 the release of the luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the  
48 pituitary gland and of testosterone or estradiol (E2) from the gonads. Opioids can also have  
49 direct effects on the pituitary gland and the testes [18].

50            Hence, the study was aimed at examining the effect of tramadol treatments on weights  
51 testes and epididymies, sperm count, and testicular integrity in male albino rat as mammalian  
52 models.

## 53 **MATERIALS AND METHODS**

### 54 **Experimental animals**

55            Eighteen healthy male albino rats of 12 weeks old; with average body weight of 150-  
56 170g were obtained from the animal house of the Department of Genetics and Biotechnology,  
57 University of Calabar, Calabar for this study. The rats were housed in well ventilated wire  
58 mesh cages under standard laboratory conditions. They were allowed free access to water and  
59 pelleted commercial feed throughout the period of the experiment. Generally, the study was  
60 conducted in accordance with the recommendations from the declarations of Helsinki on  
61 guiding principles in care and use of animals and the local ethical committee.

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63

### 64 **Experimental design and procedure**

65 The eighteen rats were divided into three groups of six rats each using a completely  
66 randomized design. The animals were acclimatized for one week before the commencement  
67 of the treatment. The daily treatments were given orally via oral gavage for 65 days and the  
68 protocol is shown in Table 1.

69 **Table 1: Protocol for daily treatment of experimental animals**

Treatment groups	Description of daily treatment
Control	No treatment. Free access to water and pelleted commercial feed
T <sub>1</sub> (50mg/kgBW)	Tramadol, 50 mg/kgBW via oral gavage. Free access to water and pelleted commercial feed
T <sub>2</sub> (100mg/kgBW)	Tramadol, 100 mg/kgBW via oral gavage. Free access to water and pelleted commercial feed

71 **LD<sub>50</sub> of tramadol = 300–350mg/kg body weight for rat and mouse [19].**

72

73 The rats were sacrificed under chloroform anaesthesia 24 hours after the last  
74 treatment. The epididymes and testes were dissected out and weighed using Scout Pro SPU  
75 601 electronic weighing balance. The epididymes were processed for epididymal sperm count  
76 while testes were processed for testicular histology.

#### 77 **Weight of testes and epididymes**

78 The epididymes and testes were dissected out and weighed using Scout Pro SPU 601  
79 electronic weighing balance.

#### 80 **Sperm count**

81 The epididymal sperm samples were obtained by macerating known weights of cauda  
82 epididymes in physiological saline in the ratio of 1:10 weight by volume, after vigorous  
83 pipetting to release the sperm cells. The suspension was filtered using an 80µm stainless  
84 mesh. Epididymal sperm count was obtained by cytometry using the improved Neubauer  
85 cytometer and was expressed as million/ml of suspension [20].

#### 86 **Histology of testes**

87 The testes were fixed in 10% formol saline. The fixed tissues were transferred to a  
88 graded series of ethanol and then cleared in xylene. Once cleared, the tissues were infiltrated  
89 in molten paraffin wax in the oven at 58°C. Serial sections of 5µm thickness were obtained  
90 from the solid block of tissue, cleared, fixed in clean slide, stained with haematoxylin and  
91 eosin stains and examined with the light microscope.

#### 92 **Statistical analysis**

93 Data from sperm count, weight of epididymes and testes were subjected to one-way  
94 analyses of variance (ANOVA) test for significant difference. Statistical significance were  
95 considered if  $p < 0.05$  while least significant difference (LSD) test was used to separate the  
96 means.

## 97 RESULTS

### 98 Weight of testes and epididymes

99 There was no significant difference in the weight of testes for the control and  
100 tramadol treated groups. The weight of epididymes reduced significantly ( $p < 0.05$ ) in rats  
101 treated with tramadol when compared with the control as shown in Table 2. Similarly, there  
102 were significant and dose related reduction in sperm count of rats treated with tramadol ( $T_1$   
103 and  $T_2$ ) when compared to the control groups.

104 **Table 2: Effect of tramadol treatments on sperm count, weight of testes and epididymes**  
105 **in albino rats**

106 Parameters	Control	$T_1$ (50mg/kgBW)	$T_2$ (100mg/kgBW)
Weight of testes (g)	1.32 <sup>a</sup> ±0.03	1.22 <sup>a</sup> ±0.04	1.23 <sup>a</sup> ±0.05
Weight of epididymes (g)	0.425 <sup>a</sup> ±0.03	0.343 <sup>b</sup> ± 0.025	0.343 <sup>b</sup> ± 0.025
Sperm count ( $\times 10^6$ /ml)	7.838 <sup>a</sup> ±0.55	6.335 <sup>b</sup> ±0.35	5.945 <sup>b</sup> ±0.20

107 Values across the table with similar superscripts are not significantly different at 5% based on  
108 ANOVA.

### 109 Sperm count

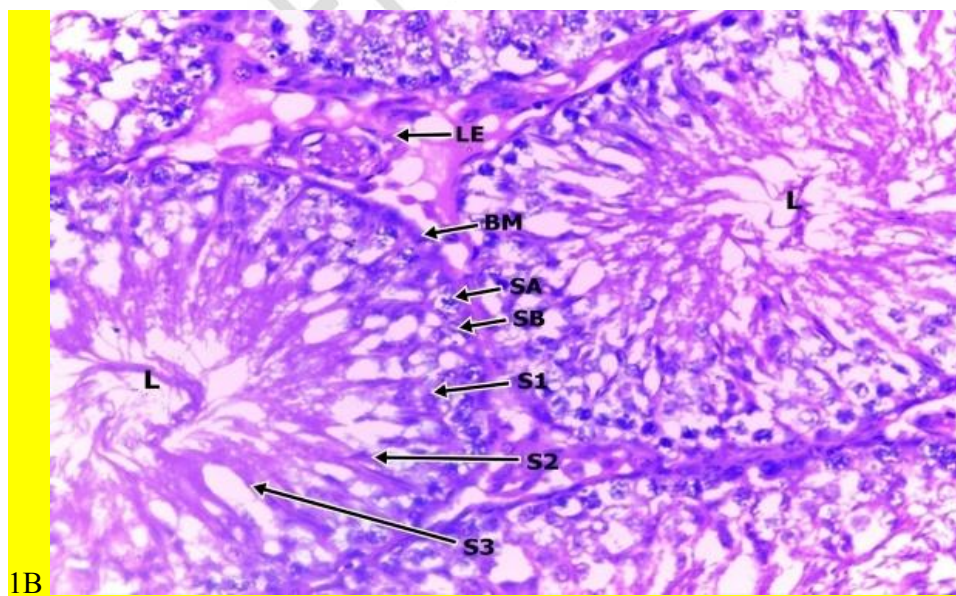
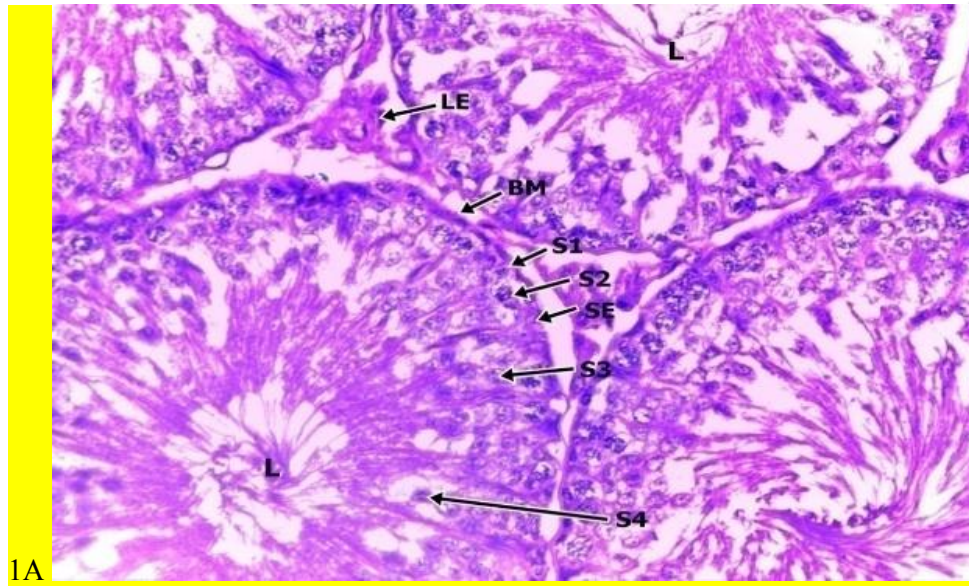
110 Similarly, there was also significant and dose related reduction in sperm count of rats  
111 treated with tramadol ( $T_1$  and  $T_2$ ) when compared to the control groups as shown in Table 2.

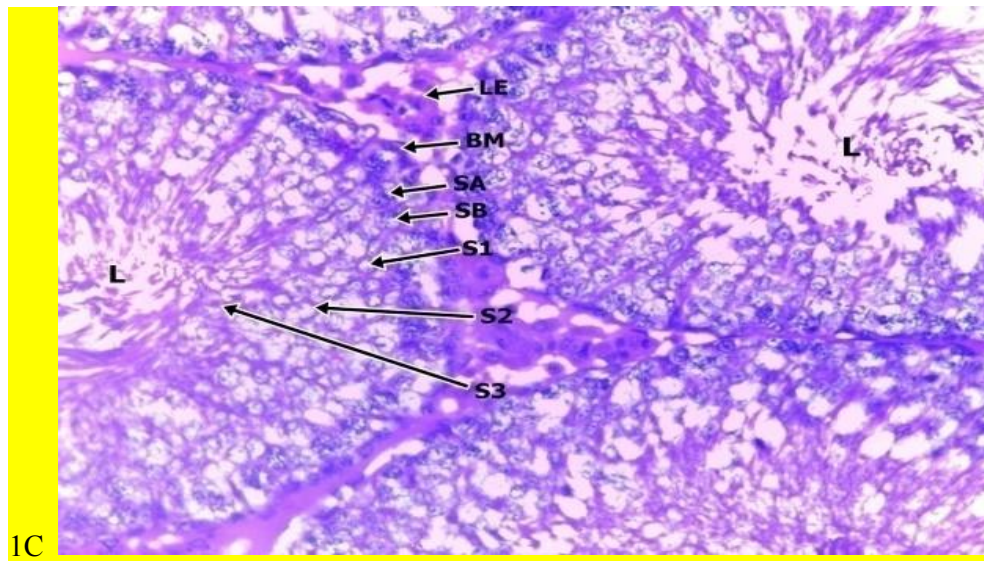
### Histology of testes

Histological examination of testicular sections in Plate 1A-C show effect of tramadol treatments on testicular integrity of rats. The rats in control group showed the basement membranes (BM) intact and prominent seminiferous tubules at different stages of development and maturation. The lumen was filled with flagella, with absence of inflammation, haemorrhage around the Leydig cells, necrosis and atrophy.

Testicular section of rats treated with  $T_1$  (50 mg/kgBW) showed prominent seminiferous tubules containing proliferating spermatogonia at various stages of maturation. The separating peritubular and intertubular interstitium was scanty containing clusters of

round to oval leydig cell with congested blood vessels showing slight atrophy and haemorrhage. While, the testicular section of rats treated with T<sub>2</sub> (100 mg/kgBW) showed prominent seminiferous tubules with intact basement layers containing spermatogonia at various stages of maturation. The lumen were filled with fewer spermatids with slight necrosis and atrophy. There was severe inflammation and haemorrhage around the leydig cells.





**Plate 1: Effect of tramadol treatments on testicular integrity of rats. (H&E X400)**

S1=Primary Spermatocyte, S2= Secondary Spermatocyte, S3=Spermatid, S4= Spermatozoa, BM= Basement membrane, SA= Spermatogonia A, SB= Spermatogonia B and L= Lumen.

**Plate 1A:** Testicular section of control rat with the basement membranes (BM) intact and prominent seminiferous tubules at different stages of development and maturation. The cells have uniform nuclei contour with coarse chromatin pattern. The lumen is filled with eosinophilic flagella.

**Plate 1B:** Testicular section of rats treated with T<sub>1</sub> (Tramadol, 50 mg/kgBW) showing prominent seminiferous tubules containing proliferating spermatogonia at various stages of maturation. The tubule contain 4 to 5 cell layer thick of spermatogonia consisting of spermatogonia A and B, primary spermatocytes, secondary spermatocytes, spermatids and spermatozoa. The cells are held together by 3 to 5 sertoli cells per tubules. The separating peritubular and intertubular interstitium is scanty containing clusters of round to oval leydig cell with congested blood vessels showing slight atrophy and haemorrhage.

**Plate 1C:** Testicular section of rats treated with T<sub>2</sub> (Tramadol, 100 mg/kgBW) showing prominent seminiferous tubules with intact basement layers containing spermatogonia at various stages of maturation. The cells are 3 to 5 cell layer thick with regular nuclei outline and fine chromatin pattern. The lumen are filled with fewer spermatids with slight necrosis and atrophy. There is severe inflammation and haemorrhage around the leydig cells.

## DISCUSSION

Results obtained revealed that tramadol treatments had significant effect on the weight of epididymes, sperm count and testicular integrity which agrees with the findings of Marwa *et al.* [21], Ceccarelli *et al.* [22] and El-Ghawet [23] who reported that tramadol caused disorganization of the seminiferous tubules with almost missing of sperm and comparatively decreased spermatogenic cells. Results obtained are also in line with previous report on the gonadotoxic effects of tramadol in male animal models by Marwa and Abdel-Malak [20] and El-Gaafarawi [24] who reported that tramadol significantly decreased sex hormones and degeneration of spermatogonia, distortion of Sertoli cell tight junctions, and accumulation of electron-dense bodies in Sertoli cells. Histological findings clearly revealed that the normal architecture and integrity of the testes of tramadol treated animals were altered causing atrophy and necrosis of the spermatogenic cells (Sertoli and Leydig cells). Sertoli cells are considered as nursery units for the developing sperms [25]. Also, Mruk and Cheng [26] reported that Sertoli cells are major supporters of spermatogenesis and germ cells because of the secretion of proteins such as core protein histone, androgen binding protein, androgen binding protein–heat shock protein 27, N-cadherin, and desmoglein. The Leydig cells play an important role in the function and structure of seminiferous tubules and in the synthesis of testosterone, which is vital for the regulation of spermatogenesis. Reduced intratesticular testosterone results in apoptosis of germ cells [27].

Issop *et al.* [28] added that steroid biosynthesis is a multistep process controlled by pituitary hormones, and this process is accelerated by the hormone dependent organelle communication network mediated by protein-to-protein interactions and inter-organelle trafficking, resulting in the efficient and timed delivery of cholesterol into the mitochondria for steroid synthesis. Therefore, reduced steroidogenesis results in altered spermatogenesis and spermatogenic failure. This could be the underlying cause of the significant reduction in the sperm count and weight of epididymes observed in tramadol treated animals. This assertion is corroborated by Ax *et al.* [29], Ezzat and el-Gohary [30], Boockfor and Blake [31], Kaukab and Saeed [32]. More so, Glover and Assinder [33] and Ekaluo *et al.* [34] reported that the distortion in fertility in male mammals is directly correlated with the disruption of spermatogenesis and the hormone regulatory mechanisms and pathways.

Studies have also shown that increased reactive oxygen species (ROS) levels and oxidative stress correlates positively with decreased sperm parameters [35-37], where it reported that oxidative stress causes significant damage to biological molecules such as lipid peroxidation, DNA damage and testicular histopathology as well as decline in sperm quality.

It has also been reported that tramadol suppresses testosterone by inducing nitric oxide (NO) [38]. A well-characterized consequence of NO compounds is the reduction in steroidogenic enzyme activities [39]. Inhibition of LH stimulated steroidogenesis may be reinforced by NO in Leydig cells [40]. Excessive NO production might inhibit the production of testicular adenosine 3, 5-cyclic monophosphate, which helps to transport cholesterol to the inner mitochondrial membrane, culminating in lower testosterone release [41]. All these reports suggest that the degenerative changes in germ cells observed in this study might be attributed to hormonal deficiency.

### **Conclusion**

The present study showed that tramadol had an adverse effect on the testicular integrity, weight of epididymes and sperm count of male albino rats in a dose-dependent pattern. Therefore, the arbitrary use of the drug should be discouraged in view of its negative effects on testicular tissues and sperm profile.

Ethical: The study was conducted in accordance with the recommendations from the declarations of Helsinki on guiding principles in care and use of animals and the local ethical committee.

Consent: NA

### **Competing interests**

Authors have declared that no competing interests exist.

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