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Histopathological Effects of Tramadol Treatments on the Testes 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

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_1 and T_2 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_1 and T_2 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_1 treated rats while severe inflammation and haemorrhage around the Leydig cells were observed in T_2 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

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epididymes (from 0.425 g in the control group to 343 g for T_1 and T_2 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.

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. Increasing and alarming rates 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 weights testes and epididymies, sperm count, and testicular integrity in male albino rat as mammalian models.

2. MATERIALS AND METHODS

2.1 Experimental Animals

Eighteen healthy male albino rats of 12 weeks old; with average body weight of 150-170 g were obtained from the animal house of the Department of Genetics and Biotechnology, University of Calabar, Calabar for this study. The rats were housed in well ventilated wire mesh cages under standard laboratory conditions. They were allowed free access to water and pelleted commercial feed throughout the period of the experiment. Generally, 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.

2.2 Experimental Design and Procedure

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

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_1 (50 mg/kgBW)	Tramadol, 50 mg/kgBW via oral gavage. Free access to water and pelleted commercial feed
T_2 (100 mg/kgBW)	Tramadol, 100 mg/kgBW via oral gavage. Free access to water and pelleted commercial feed

LD_{50} of tramadol = 300–350 mg/kg body weight for rat and mouse [19]

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

2.3 Weight of Testes and Epididymes

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

2.4 Sperm Count

The epididymal sperm samples were obtained by macerating known weights of cauda 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 80µm stainless mesh. Epididymal sperm count was obtained by cytometry using the improved Neubauer cytometer and was expressed as million/ml of suspension [20].

2.5 Histology of Testes

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

2.6 Statistical Analysis

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

3. RESULTS

3.1 Weight of Testes and Epididymes

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

3.2 Sperm Count

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

3.3 Histology of Testes

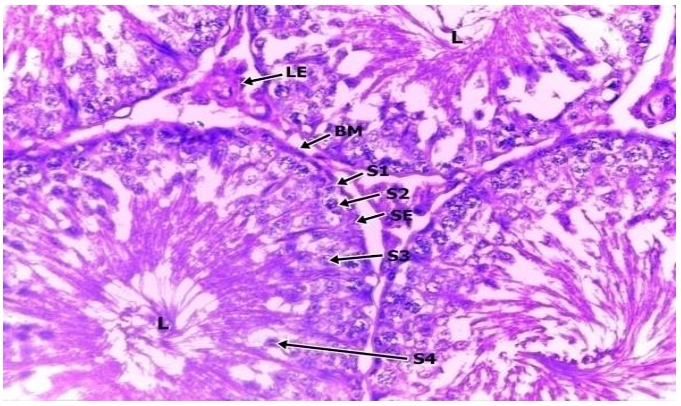
Histological examination of testicular sections in Plate 1A-C show effect of tramadol treatments on testicular integrity of rats. The rats in control group showed the basement membranes (BM) intact and prominent seminiferous tubules at different stages of development and maturation. The lumen was filled 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_2 (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.

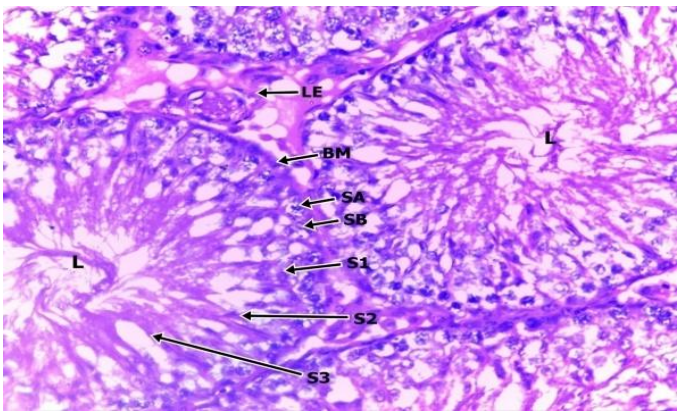
Table 2. Effect of tramadol treatments on sperm count, weight of testes and epididymes in albino rats

Parameters	Control	T_1 (50 mg/kgBW)	T_2 (100 mg/kgBW)
Weight of testes (g)	1.32^{a}±0.03	1.22^{a}±0.04	1.23^{a}±0.05
Weight of epididymes (g)	0.425^{a}±0.03	0.343^{b}±0.025	0.343^{b}±0.025
Sperm count ($x10^{6}$/ml)	7.838^{a}±0.55	6.335^{b}±0.35	5.945^{b}±0.20

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



(1A)



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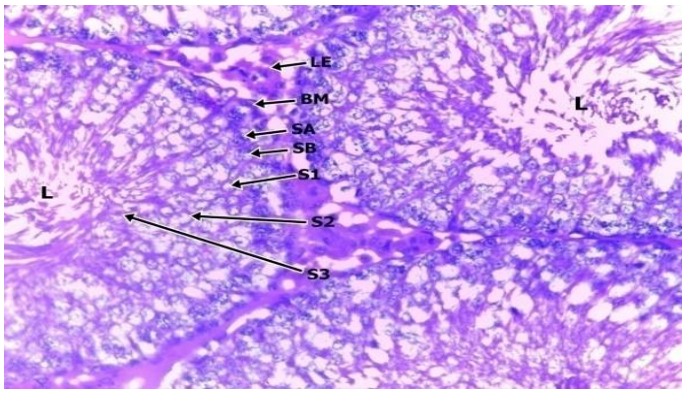

(1C)

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_1 (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_2 (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.*

4. 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 intra-testicular 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 spermatic 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.

5. 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 APPROVAL

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.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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