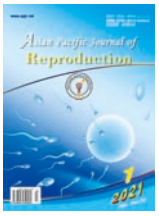

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Therapeutic levels of short-term tramadol administration negatively affect testis function in rats

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ABSTRACT

Objective: To investigate the effects of 30-day treatment with therapeutic dose equivalent levels of tramadol on serum testosterone level, sperm parameters, and testicular histology in rats.

Methods: Thirty-five Wistar rats were equally divided into seven groups. Group 1 (the control group) received distilled water (0.5 mL) daily for 30 days. Groups 2-4 were gavaged with therapeutic dose equivalent levels of tramadol (1.25, 2.50 and 5.00 mg/kg/day body weight, respectively) in two equal divided doses for 30 consecutive days, and sacrificed on day 31. Groups 5-7 received similar tramadol treatments as above but they were allowed for another 30 days to recover after receiving the last dose and sacrificed on day 61 for reversibility study. Serum testosterone level and epididymal sperm were analyzed, and histopathological examination of the testis was carried out.

Results: Tramadol treatment significantly decreased serum testosterone levels compared with the control group. Furthermore, tramadol treatment inhibited sperm motility and significantly and dose-dependently decreased sperm count and viability compared with the control group. In addition, tramadol significantly increased morphological abnormalities in sperm (P<0.05). The above effects of tramadol were reduced in the reversible groups. Testis histopathological examination revealed disintegrated cell architecture, eroded and atrophied seminiferous tubules, and a marked decrease in the number of spermatogenic cells in the tramadol treated groups. The histopathological changes were restored in the reversible groups, but improvement was not complete in the 5.00 mg/kg tramadol treated reversible group.

Conclusions: Long term treatment with tramadol at clinical dose levels may adversely affect testosterone level, sperm parameters, and testicular histology, but they are reversible at lower doses.

KEYWORDS: Tramadol; Testosterone; Sperm parameters; Opioid analgesic; Testicular function

1. Introduction

Tramadol is a synthetic opioid analgesic used clinically for the treatment of moderate to severe pains[1-3]. Tramadol mechanism of action involves activation of μ-opioid receptors in the central nervous system, as well as inhibition of the reuptake of serotonin and norepinephrine[4-6]. The drug is very useful for the above indication, but inappropriate or frequent use is unhealthy as it has dependence potential among other unpleasant consequences[7,8]. Unfortunately, misuse and abuse of tramadol is prevalent among youths in many countries, including Nigeria[9-11]. This situation, which is partly encouraged by poor drug control systems and easy drug accessibility, has resulted in serious adverse medical and social problems. Aside from the common adverse effects like constipation, nausea and vomiting, dizziness, confusion, and hallucination, tramadol has been associated with hazardous effects on different body organs and systems[12-15].

Tramadol abuse in males raises concern of its potential effect on the testis. Neuropharmacologic agents that stimulate or inhibit the central nervous system activities can modify the hypothalamic-pituitary control of the testis[16,17]. Also, alteration of hypothalamic function or pituitary gonadotropins secretion (directly or indirectly) by drugs can substantially affect normal functioning of the male reproductive system[18,19]. Thus, it is

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logical to imagine or hypothesize that tramadol intake over a long period of time may negatively affect reproductive parameters in young males. This concern is increased with the recent finding that tramadol is helpful in premature ejaculation in humans as an off-label application[10,20,21], which is related to its serotonin re-uptake inhibiting properties[22,23]. The above would certainly increase the frequency of tramadol usage and promote its abuse among young males because of the sexual performance enhancement and euphoric effect they derive from it. Although this "on demand" therapy for premature ejaculation of tramadol appears to make the drug therapeutically attractive, its influence on testicular activity or male fertility remains a significant concern, particularly in view of the rising rate of male infertility[24].

A number of studies have reported adverse effects on different components of the male reproductive system following tramadol treatment[16,25-30]. Some studies have reported that tramadol altered reproductive hormone levels, including testosterone[16,25-27,30], and also caused damage to testicular tissue and associated oxidative stress when administered over a period of time in rats[28-30]. Besides the above reported testicular effects of tramadol, long-term use of tramadol is believed to significantly affect male sexual dysfunction[10,17]. However, the doses of tramadol applied in most of the above studies were higher than therapeutic daily dose levels of the drug. Therefore, knowledge on the likely outcome of prolong treatment with standard clinical daily dose equivalents of the drug (25-400 mg in adult human) would be of clinical relevance. Such information would not only be useful to guide in making rational decisions, but also help in taking the right precautions when prescribing tramadol for patients for chronic medical conditions. To address the above concern, this study investigated the testicular outcome in Wistar rats after oral administration of equivalent daily therapeutic dose levels of tramadol consecutively for 30 days by analyzing serum testosterone level, sperm parameters, and testicular histology. Additionally, we evaluated whether the outcome observed would be reversible.

2. Materials and methods

2.1. Animals

Thirty-five healthy male Wistar rats weighing 180-200 g and age range of 12-13 weeks were used for the experiment. The animals were obtained from the Animal House of the Faculty of the Pharmaceutical Sciences, University of Port Harcourt, Nigeria. They were housed in separate wire mesh cages (50 cm × 80 cm) to avoid overcrowding, and acclimated for 14 days before used for experiment. The animals were throughout maintained at a room temperature of (24-28) ℃ and natural lighting condition, and allowed access to rodent pellets and water freely (as per standard feeding). Animals were handled according to standard international guidelines[31]. Experiment was carried out in November, 2018.

2.2. Study design

The rats were divided into seven groups (n=5 per group). Group 1 served as the control group and was administered distilled water (0.5 mL) daily for 30 days. Groups 2, 3, and 4 were administered 1.25, 2.50 and 5.00 mg/kg body weight tramadol (Zintex Technologies Ltd., London) daily, respectively in two equal divided doses for 30 days and sacrificed on day 31. Groups 5, 6 and 7 were treated as the animals above but they were allowed for another 30 days to recover after receiving the last dose and sacrificed on day 61 for reversibility study. The doses were derived based on the daily therapeutic dose range of tramadol in humans reported in previous studies[32,33]. All the administrations were done by oral gavage using an orogastric syringe. The drug suspension and distilled water were administered in the morning and evening daily before rats were fed. The rats were anesthetized with diethylether and sacrificed on the appropriate scheduled dates. Blood samples (about 3 mL) were collected in the evening during animal sacrifice by cardiac puncture into sterile syringe and allowed to clot. The blood samples were then centrifuged at 4 500 ×g at room temperature for 20 min and serum was separated for evaluation of testosterone level. Testosterone concentration was obtained by enzyme linked immunosorbent assay technique using commercially available kits (Biocheck Inc., South San Francisco, CA, USA). Also, the abdominal region of the rats were dissected and the epididymis was isolated and used for sperm analysis, while the testis was fixed in 10% formal saline for histopathological examination.

2.3. Analysis of sperm parameters

Excised caudal epididymis was placed in a petri dish containing sodium bicarbonate buffered Tyrode's solution, and incised at several points with sharp sterile blade and sperm was gently drawn into plastic transfer pipette. Sperm suspensions were prepared and viewed under the microscope and sperm parameters (motility, count, viability and morphology) were estimated by using standard procedures[34]. Briefly, to evaluate sperm motility, undiluted sperm was placed on a glass slide and covered with a cover slip and viewed under the microscope (Surgifield Medicals, UK) immediately after sperm collection. Immotile and motile sperm were counted in at least 10 randomly selected fields using 400× magnification, and values were expressed as percentages. Sperm motility was graded as: progressively motile, non-progressively motile, and immotile sperm. Sperm count was obtained by using the Neubauer hemocytometer chamber. Sperm was mixed with diluting fluid (bicarbonate-formalin) and the resulting sperm suspension was placed in the Neubauer counting chamber and allowed to settle for 15 min. Complete morphologically mature sperm were then counted by using 400× magnification. For sperm viability, a drop of sperm was placed on a slide and one drop of 0.5% eosin stain was applied and left for about 2 min at room temperature. The number of stained and unstained spermatozoa was recorded when the slide was examined

under the microscope. The unstained spermatozoa were considered viable and expressed as a percentage of the total sperm counted. For sperm morphology, sperm smears were stained on microscopic slides with two drops of Walls and Ewas after air-drying. Morphological characteristics were examined under bright field optics (Surgifield Medicals, UK) at 1 000× magnification with oil immersion. About one hundred spermatozoa were counted and sperm morphology was expressed as percentages of sperm with normal morphology and sperm presenting with abnormalities in head, neck, and tail.

2.4. Histopathological examination of testis

Testis samples were processed following standard histological techniques[35]. Briefly, testis tissue was fixed in 10% formal saline for about 5 days, dehydrated in graded concentrations of alcohol, cleared or defatted in xylene, impregnated with soft paraffin wax, embedded in molten wax, blocked out, sectioned (5-7 μm thickness) with a microtome, and stained with hematoxylin and eosin (H & E) in successive order. The slide was viewed under light microscope (Nikon Eclipse E400) and analyzed for pathology and relevant sections were photographed using 400× objective.

2.5. Statistical analysis

The data obtained for sperm parameters and testosterone were analyzed by using GraphPad Prism 5 Software (GraphPad Software Inc., San Diego, USA). Data samples were tested for normality and comparisons between the control and experimental groups were performed by using one way analysis of variance followed by Neuman-keuls post test. When data were not distributed normally, analyses were performed by using Kruskal-Wallis test and differences from controls were identified by Dunn test. In all analyses, values were considered to be significant at $P<0.05$, and data were expressed as mean±standard deviation (mean±SD).

2.6. Ethics statement

All experimental procedures were reviewed and approved by the Ethics Committee of the University of Port Harcourt, Nigeria (UPH/CHREC/APP/076/2017).

3. Results

3.1. Serum testosterone levels

Serum testosterone concentration was significantly reduced in treated groups (groups 3 and 4) in a dose-dependent manner as compared with the control group (Figure 1). Testosterone levels in the reversible groups were not significantly different when compared with the control group (Figure 1).

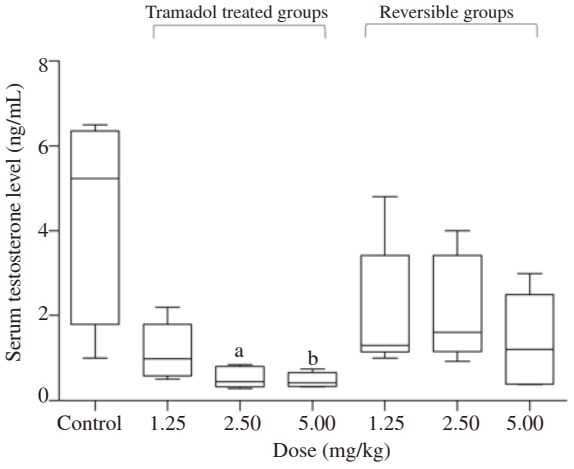

Figure 1. Serum testosterone levels in Wistar rats following 30-day oral administration of therapeutic dose equivalent levels (1.25, 2.50 and 5.00 mg/kg) of tramadol. Tramadol treated groups are sacrificed at the end of tramadol treatment, while reversible groups are allowed to recover for 30 days before sacrificed. Values are expressed as median (interquartile range) [median (IQR)]; $n=5$ animals per group. [a]$P<0.05$, [b]$P<0.01$: *versus* the control group. Data are analysed by using Kruskal-Wallis test and Dunn post test.

3.2. Sperm parameters

The number of sperm with progressive motility in the 2.50 and 5.00 mg/kg tramadol treated groups was both significantly decreased (Table 1), while the number of sperm with non-progressive motility was not significantly affected as compared with the control group in neither of the two groups (Table 1). In addition, immotile sperm population was significantly increased in 2.50 and 5.00 mg/kg tramadol treated groups, but it was not affected in the group that received 1.25 mg/kg as compared with the control group (Table 1). In the reversible groups, the decreased progressive motility in 1.25 and 2.50 mg/kg tramadol treated groups were restored after 30 days recovery, and there were no significant difference between the control group and the 1.25 and 2.50 mg/kg treated reversible groups. But progressive motility in the 5.00 mg/kg tramadol reversible group was significantly lower than the control group (Table 1). Further, non-progressive sperm motility in all reversible groups were not significantly different from that of the treated groups (Table 1), whereas immotile sperm population of 2.50 and 5.00 mg/kg recovery groups were reduced comparing with the treated groups (Table 1).

Sperm viability (number of viable sperms) was significantly and dose-dependently decreased in the tramadol treated rats as compared with the control group (P all<0.05) (Table 1), but the decreased sperm viability were restored in the reversible groups except the 5.00 mg/kg tramadol reversible group which was significantly lower than that of control group ($P=0.018$) (Table 1).

Table 1. Sperm motility and viability in Wistar rats following 30-day oral administration of therapeutic dose equivalent levels (1.25, 2.50, 5.00 mg/kg) of tramadol.

Parameters	Control	Tramadol treated groups			Reversible groups		
		1.25 mg/kg	2.50 mg/kg	5.00 mg/kg	1.25 mg/kg	2.50 mg/kg	5.00 mg/kg
Progressive motile sperm (%)	50.80±5.81	36.20±10.71	17.60±7.50^{a**}	9.00±4.18^{a**}	47.20±11.69	48.40±10.50^{b**}	30.40±6.15^{a*c*}
Non-progressive motile sperm (%)	25.60±6.69	25.00±10.00	12.00±5.70	11.00±5.43	25.80±11.58	24.00±6.52	27.00±10.37
Immotile sperm (%)	23.60±3.91	42.80±8.76	70.40±5.83^{a**}	80.00±9.35^{a**}	27.00±5.83	27.60±8.88^{b**}	42.60±15.19^{c**}
Sperm viability (%)	51.80±6.06	33.60±15.53^{a}	27.60±11.10^{a*}	20.00±7.91^{a**}	42.40±10.06	41.40±6.07	31.20±5.98^{a}

Values are expressed as mean±SD; n=5 animals per group. ^{a}P<0.05 *versus* the control group, $^{a*}P$<0.01 *versus* the control group, $^{a**}P$<0.001 *versus* the control group; $^{b**}P$<0.001 *versus* the 2.5 mg/kg tramadol group; $^{c*}P$<0.01 *versus* the 5.00 mg/kg tramadol group, $^{c**}P$<0.001 *versus* the 5.00 mg/kg tramadol group. The tramadol treated groups are sacrificed at the end of tramadol treatment, while the reversible groups are allowed to recover for 30 days before being sacrificed.

Table 2. Sperm count and morphology in Wistar rats following 30-day oral administration of therapeutic dose equivalent levels (1.25, 2.50, 5.00 mg/kg) of tramadol.

Parameters	Control	Tramadol treated groups			Reversible groups		
		1.25 mg/kg	2.50 mg/kg	5.00 mg/kg	1.25 mg/kg	2.50 mg/kg	5.00 mg/kg
Sperm count ($\times 10^6$/mL)	96.40±25.75	35.40±15.87^{a*}	25.60±9.24^{a**}	18.40±2.88^{a**}	90.80±25.41^{b*}	65.00±29.81^{c}	53.40±24.65^{a}
Sperm with normal morphology (%)	72.00±21.68	42.40±5.13^{a*}	35.00±10.00^{a**}	25.20±7.92^{a**}	65.40±16.15^{b}	61.40±6.69^{c}	61.00±8.94^{d**}
Sperm with abnormal morphology (%)	28.00±13.04	57.60±5.53^{a*}	65.00±8.25**	74.80±10.62^{a**}	34.60±16.38^{b}	38.60±5.73^{c}	39.00±10.25^{d**}

Values are expressed as mean±SD; n=5 animals per group. ^{a}P<0.05 *versus* the control group, $^{a*}P$<0.01 *versus* the control group, $^{a**}P$<0.001 *versus* the control group; ^{b}P<0.05 *versus* the 1.25 mg/kg tramadol group, $^{b*}P$<0.01 *versus* the 1.25 mg/kg tramadol group; ^{c}P<0.05 *versus* the 2.50 mg/kg tramadol group; $^{d**}P$<0.001 *versus* the 5.00 mg/kg tramadol group. The tramadol treated groups are sacrificed at the end of tramadol treatment, while the reversible groups are allowed to recover for 30 days before being sacrificed.

Sperm count was significantly decreased in all tramadol treated rats when compared to the control group (Table 2). In the reversible groups, sperm counts were increased as compared with the treated groups, and the values were comparable to the control group except the 5.00 mg/kg tramadol group which was significantly reduced (P=0.016) (Table 2). Sperm morphology in tramadol administered rats was affected negatively as sperm with normal morphology was significantly decreased, and those with abnormal morphology (abnormal head, neck, and tail) were significantly increased as compared with the control group (Table 2). In the reversible groups, the percentage of sperm with normal morphology was significantly increased compared with tramadol administered rats, while those with abnormal morphology were significantly decreased, but percentage of sperm with abnormal morphology was not significantly increased as compared with the control group (Table 2).

3.3. Testis histopathological findings

The testes of the control rats showed normal seminiferous tubules with well differentiated spermatogenic cells and normal Leydig cell (Figure 2A). Compared with the control group, there was dose-dependent alterations in the histology of rats that received tramadol, characterized by atrophied seminiferous tubules in the 1.25 and 2.50 mg/kg groups (Figures 2B and 2C), and disintegrated testicular cell architecture in the 5.00 mg/kg tramadol group (Figure 2D). In reversible groups, the testes of rats that received 1.25 or 2.50 mg/kg tramadol showed normal histology of testis like the control group (Figures 2E and 2F), but few histological changes were observed in the group that received 5.00 mg/kg tramadol (Figure 2G).

4. Discussion

We observed that tramadol caused reduction in serum testosterone levels in the animals dose-dependently. This is in agreement with previous studies, which reported reduced serum testosterone concentration following 30-day oral treatment with tramadol at higher daily concentrations of between 25 to 80 mg/kg[25,27]. Similar results were obtained by Ahmed and Kurkar[16] and Salah *et al*[30] who reported that subcutaneous injection of 40 or 60 mg/kg tramadol three times a week for 8 weeks or 45 days altered reproductive hormone levels in plasma, including testosterone in rats. Additionally, previous studies reported that tramadol also affected other reproductive hormones, such as gonadotropins, and prolactin[16,30,36,37]. Normally, testosterone is secreted by the Leydig cell in the testis through the direct stimulation of the gonadotropic hormone (luteinizing hormone) of the anterior pituitary gland, which is in turn stimulated by gonadotropic hormone releasing hormone (GnRH) of the hypothalamus[18,38,39]. When secreted, testicular level of testosterone is regulated through negative feedback control mechanism which involves the hypothalamus and pituitary, such that elevation in testosterone level sends an inhibitory signal which causes a reduction in luteinizing hormone and GnRH secretions, and vice versa[18,39]. Some of the previous studies have suggested that testosterone reduction by tramadol may involve interference of this hypothalamic-pituitary regulatory function[16,30,37].

Besides the reduction of sperm count, the results of this study also demonstrated a significant association between tramadol administration and impaired quality of sperm in the rats. Tramadol treatment reduced sperm viability, lowered sperm motility and induced structural abnormalities particularly at 2.50 and

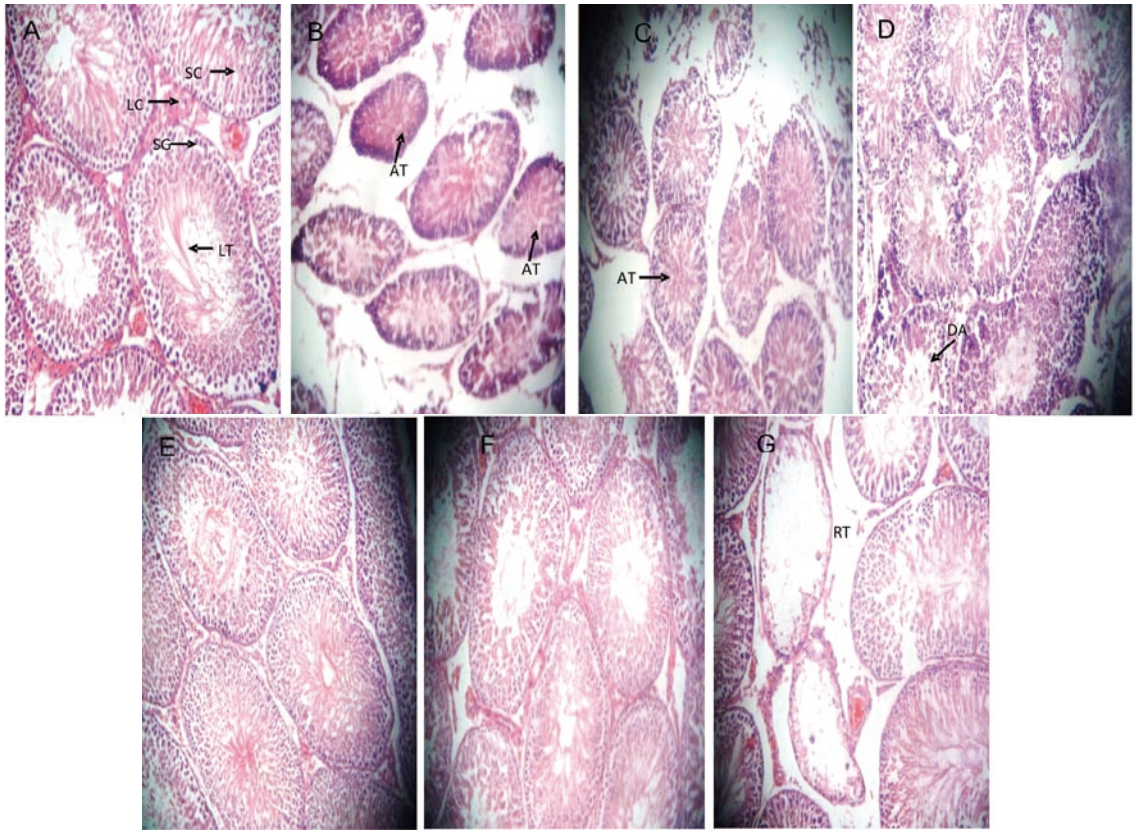

Figure 2. Photomicrographs showing effects of 30-day oral administration of therapeutic dose equivalent levels of tramadol on testis of Wistar rats (H & E, magnification ×100). A (Group 1, the control group): the normal lumen of seminiferous tubules (LT) with spermagonium (SG), other spermatogenic cells (SC) and Leydig cell (LC). B (Group 2, received 1.25 mg/kg/day tramadol): histology characterized with atrophied seminiferous tubules (AT). C (Group 3, received 2.50 mg/kg/day tramadol): shows histology characterized with atrophied seminiferous tubules (AT). D (Group 4, received 5.00 mg/kg/day tramadol): disintegrated tissue architecture (DA). E (Group 5, received 1.25 mg/kg/day tramadol and allowed to recover for 30 days): normal histology of testis like the control group. F (Group 6, received 2.50 mg/kg/day tramadol and allowed to recover for 30 days): normal histology of testis like the control group. G (Group 7, received 5.00 mg/kg/day tramadol and allowed to recover for 30 days): shows reduced spermatozoa in tubular lumen (RT).

5.00 mg/kg/day, thereby diminishing sperm quality of the rats. Interestingly, histopathological alterations like erosion and atrophy of seminiferous tubules were equally observed after treatment with tramadol in the current study. Similar reports had indicated that long term administration of tramadol caused various forms of histopathological changes in experimental animals but at daily dose levels that were higher than the doses used in the present study[28–30,40,41]. Disorganization of seminiferous tubules with apoptosis of spermatogenic cells in rats by tramadol at 40 mg/kg was reported by El-Ghawet[40]. Equally, Abdellatief *et al*[41] reported that degenerative changes in seminiferous tubules like shrinkage, separation of tubular basement membrane, disorganization and vacuolization of spermatogenic layers were detected in rats treated with 40 mg/kg tramadol. The authors also reported that tramadol treatment additionally induced ultrastructural abnormalities in all spermatogenic cells and Sertoli cells. The observed histopathological changes in the testis after treatment with tramadol in the current study indicate that tramadol can potentially cause damage to both germ cells and the supporting Sertoli cells, which is supported by the results of the above cited studies. Both cells in the testis, and testosterone are indispensable for normal spermatogenesis, and alteration of their activities by tramadol will eventually affect spermatozoa production. The inhibition of sperm properties by tramadol observed in this study may therefore not be surprising. Additionally, our sperm results were not different from other previous studies that reported similar impairment of sperm count, motility and vitality by long term tramadol treatment[16,28–30]. However, the current results importantly indicate that tramadol treatment could induce adverse effects on sperm properties at therapeutic dose levels. This novel observation is very relevant in view of the fact that clinical application of tramadol for pain conditions is common among many patients. Consequently, in male patients that may require tramadol analgesic for chronic medical conditions, prescribers may have to consider its potential negative reproductive effects besides the known adverse effects. To expand on the present novel finding, more elaborate studies are suggested to understand the mechanisms involved, including investigations to determine the safe and effective minimum daily dose of tramadol.

Furthermore, the negative effects of tramadol on most of the indices evaluated were reversed after drug withdrawal for thirty days. This was true because the induced negative effects of tramadol were either reduced in intensity or completely returned to normal conditions in the reversible animals, especially among those that received the lower doses of 1.25 and 2.50 mg/kg tramadol. Recovery of tramadol induced testicular histological abnormalities with associated oxidative stress was reported by Ghoneim *et al*[29] in rats. The authors reported that recovery of the adverse histopathological effects occurred in rats that received 50 mg/kg daily treatment of tramadol for 4 weeks following 4 weeks withdrawal of drug treatment, which supports our results. The study of Ghoneim *et al*[29] equally reported that recovery of the testis tissue did not completely return to normal, which is in agreement with our results. In another study, recovery of tramadol-induced reduction of plasma testosterone level in rats was also reported by El-Gaafarawi[25], who reported that reduction in testosterone level was normalized in the rats that received 40 mg/kg tramadol daily for 30 days after 10-day withdrawal of the drug, which was consistent with our observation as elevation of testosterone levels were reversed in reversible groups in the present study. El-Gaafarawi[25] reported additionally that 80 mg/kg/day tramadol treatment produced similar adverse effects which persisted even after the 10-day recovery period, suggesting that tramadol is capable of inhibiting testosterone irreversibly at high dose levels.

The present study did not sufficiently evaluate the reproductive parameters (including hormone spectrum) and the mechanism of toxicity, so more elaborate studies may be necessary to fully establish the present findings.

In conclusion, long term treatment with tramadol at clinical dose levels may result in reduction in testosterone and histomorphological changes, as well as alteration in sperm parameters. Additionally, the adverse testicular effects are reversible at lower doses, but persistent at higher doses.

Conflict of interest statement

The authors declare that there is no conflict of interest.

Authors' contributions

Jonah Sydney Aprioku conceived and designed the study. Benjamin Toochukwu Okpe and Doupere Ben handled the literature searches, and contributed in conduction of the experiments and collection of data. Jonah Sydney Aprioku handled analysis of the data, wrote the protocol and first draft of the manuscript. All authors approved the final manuscript.

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