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The effect of coadministration of vitamin D and tramadol on serum kisspeptin, testosterone, oxidative stress levels and testicular histology in Wistar rats: a preliminary report

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Abstract

Background: Tramadol, an opioid analgesic, is known to induce testicular damage and impair reproductive parameters. Vitamin D3, recognized for its antioxidant and protective properties, might offer a potential protective effect against tramadol-induced testicular damage. This study observed the effects of co-administration of vitamin D3 and tramadol on serum kisspeptin levels, testicular histology, semen parameters, testosterone levels, and oxidative stress markers in male rats. **Methods**: Fifteen male rats weighing between 150 and 250 g were randomly divided into three groups (n = 5 per group). Group A was the control, receiving only distilled water. Group B was administered 20 mg/kg body weight of tramadol daily, while group C received both 20 mg/kg body weight of tramadol and 25 μg/kg body weight of vitamin D3 daily. The treatments were administered orally for 14 days. Post-administration body weight, relative testicular weight, serum kisspeptin levels, semen parameters, testosterone levels and oxidative stress markers (catalase, glutathione and malonaldehyde) were measured. Testicular histology was also examined using photomicrography. **Results**: No significant differences were observed in body weights, relative testicular weights, serum kisspeptin levels, semen parameters, testosterone levels, or oxidative stress markers among the experimental groups ($p > 0.05$). Histological analysis in the tramadol-treated group exhibited significant degradation of spermatozoa, which was not mitigated by vitamin D3 co-administration compared to the control group. **Conclusions**: The study demonstrates that vitamin D3 supplementation does not significantly ameliorate tramadol-induced testicular damage. There is a need for further research with varied doses and longer durations to further explore the potential protective mechanisms of vitamin D3.

Keywords

Tramadol; Vitamin D; Semen analysis; Reproductive toxicity; Oxidative stress; Kisspeptin; Testosterone

Efecto de la coadministración de vitamina D y tramadol en los niveles de kisspeptina, testosterona, estrés oxidativo en suero y la histología testicular en ratas Wistar: un informe preliminar

Resumen

Antecedentes: El tramadol, un analgésico opioide, es conocido por inducir daño testicular y afectar parámetros reproductivos. La vitamina D3, reconocida por sus propiedades antioxidantes y protectoras, podría ofrecer un efecto protector potencial contra el daño testicular inducido por tramadol. Este estudio observó los efectos de la coadministración de vitamina D3 y tramadol en los niveles séricos de kisspeptina, la histología testicular, los parámetros de semen, los niveles de testosterona y los marcadores de estrés oxidativo en ratas macho. **Métodos**: Quince ratas macho con un peso entre 150 y 250 g fueron divididas aleatoriamente en tres grupos (n = 5 por grupo). El Grupo A fue el control, recibiendo solo agua destilada. Al Grupo B se le administró 20 mg/kg de peso corporal de tramadol diariamente, mientras que el Grupo C recibió tanto 20 mg/kg de peso corporal de tramadol como 25 μg/kg de peso corporal de vitamina D3 diariamente. Los tratamientos se administraron por vía oral durante 14 días. Se midieron el peso corporal posterior a la administración, el peso testicular relativo, los niveles séricos de kisspeptina, los parámetros de semen, los niveles de testosterona y los marcadores de estrés oxidativo (catalasa, glutatión y malonaldehído). También se examinó la histología testicular mediante fotomicrografía. **Resultados**: No se observaron diferencias significativas en el peso corporal, el peso testicular relativo, los niveles séricos de kisspeptina, los parámetros de semen, los niveles de testosterona o los marcadores de estrés oxidativo entre los grupos experimentales ($p > 0.05$). El análisis histológico en el grupo tratado con tramadol mostró una degradación significativa de los espermatozoides, que no fue mitigada por la coadministración de vitamina D3 en comparación con el grupo control. **Conclusiones**: El estudio demuestra que la suplementación con vitamina D3 no mejora significativamente el daño testicular inducido por tramadol. Es necesario realizar más investigaciones con dosis variadas y duraciones más prolongadas para explorar mejor los posibles mecanismos protectores de la vitamina D3.

Palabras Clave

Tramadol; Vitamina D; Análisis de semen; Toxicidad reproductiva; Estrés oxidativo; Kisspeptina; Testosterona

1. Introduction

Tramadol is a widely used analgesic for acute or moderately severe chronic pain treatment and management [1]. Aside from pain relief, previous studies have shown that tramadol is effective in the treatment of premature ejaculation compared with placebo or alternative therapies [2, 3]. Tramadol abuse, mostly among men [4], has been steadily increasing in recent years; reasons could be associated with the urge to augment sexual drives, the desire to achieve a harder and longer-lasting erection, the urge for higher coital frequency and the willingness to delay ejaculation [2].

Furthermore, research has shown that prolonged use of opioids like tramadol, whether intentional or unintentional, can lead to severe liver failure and respiratory arrest [5]. Its abuse has also been linked to incidents of violence and fights, road accidents, self-inflicted unintentional injuries [6], confusion, dizziness, seizures and drowsiness [7]. Other adverse effects on the reproductive system include a decrease in sexual activity, a drop in the production of follicle-stimulating hormone (FSH), testosterone and hypothalamic-pituitary function abnormalities [8, 9]. These effects encompass hormonal imbalance, oxidative stress, and direct toxicity to reproductive organs, which can lead to impaired fertility [10, 11].

Today, infertility is recognized as a common social problem, and nearly 40% of infertility problems are reported in men [12]. Reduced levels of sexual hormones, low sperm counts, abnormal sperm morphology and lack of sperm motility are the main causes of male infertility [13]. Researchers have reported that chronic tramadol use adversely affects the male reproductive tissues by altering testicular histology, disrupting spermatogenesis, causing sperm cell membrane damage, reducing testosterone levels and inducing apoptosis in interstitial cells of the testis [4, 8, 14].

Kisspeptin, a hypothalamic peptide coded by the *Kisspeptin 1* (*KiSS1*) gene [15], is critical in the release of gonadotropin-releasing hormone (GnRH), which in turn regulates the secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH), which are key hormones in testosterone production and spermatogenesis [16]. Vitamin D3, a fat-soluble vitamin, has been known to improve many reproductive processes, such as spermatogenesis, and acrosome reactions, and correlates with sperm quality [17]. Recent studies have highlighted the potential of vitamin D3 in modulating reproductive functions and protecting against oxidative stress [18]. Studies have shown that vitamin D3 improves hormonal balance, including testosterone levels and influences the expression of genes involved in antioxidant defense [19].

Therefore, the primary objective of this study is to evaluate the biochemical and testicular histopathological effects of vitamin D3 supplementation on serum kisspeptin levels, testicular histology, semen parameters, testosterone levels and oxidative stress markers in male rats following exposure to tramadol. By examining these parameters, the study aims to elucidate the potential role of vitamin D3 against tramadol-induced reproductive toxicity and the associated mechanisms of action.

2. Material and methods

2.1 Study location and duration

The study was carried out in the Anatomy Research Laboratory and the Molecular Diagnostics and Research Laboratory, College of Health Sciences, Nnamdi Azikiwe University and lasted about three months.

2.2 Drugs and animal procurement and handling

Fifteen (15) male Wistar rats were procured from an animal farm at Nnamdi Azikiwe University and acclimatized for 2 weeks under standard housing conditions (ventilated room with 12 h/12 h light/dark cycle, at 27 °C ± 2 °C normal temperature). The sample size was determined using resource equation [20]. The rats were fed *ad libitum* with water and standard rat chow throughout the experimental period. Tramadol B.P. 50 mg (NAFDAC Number: A4-0678; Mancare Pharmaceuticals, Maharashtra, India) and vitamin D3 25 mcg (Item No: 572310; Nature Made Nutritional Products, West Hills, CA, USA) were sourced from GozGreen Pharmacy Limited, Onitsha, AN, Nigeria.

2.3 Experimental design

Fifteen (15) rats weighing between 150–250 g were randomly divided into three (3) groups (A–C) of five (5) rats each. Group A received only distilled water daily and served as the control group. Group B received only 20 mg/kg.bw of tramadol daily while group C received 20 mg/kg.bw of tramadol and 25 μg/kg.bw of vitamin D3 daily. The administration of tramadol and vitamin D3 lasted for 14 days in line with a previous study [21] and were done by oral route using distilled water as vehicle and with the aid of a syringe and a cannula. All the animals were fed with normal rat chow and water throughout the experimental period. The administration doses for vitamin D3 and tramadol were based on a previous study [22].

2.4 Animal sacrifice and sample collection

The rats were sacrificed the day following the last day of administration. The animals were euthanized by cervical dislocation and blood was collected by orbital puncture for biochemical and hormonal analysis. Animals were anaesthetized intraperitoneally with pentobarbital-based solution (Euthasol: 390 mg Pentobarbital/50 mg Phenytoin/mL) at 100 mg/kg prior to euthanizing them with cervical dislocation. The blood was collected in plain tubes and were kept standing for 5 min before centrifugation, and the sera were separated later and stored at −20 °C for further analyses. The rat scrotal area was carefully exposed to harvest the testes following the incision of the scrotum. Each testis harvested was weighed and fixed in a freshly prepared Bouin's fluid. The relative testicular weights for each animal were determined by dividing the organ weight with the body weight and multiplying by a factor of 100. The epididymis was separated, and the seminal fluid collected from the caudal part for the assessment of sperm count, motility and morphology.

2.5 Histopathological tissue processing

The testicular tissue was fixed in Bouin's fluid, dehydrated in alcohol, cleared in xylene and embedded in paraffin. The tissue processing protocol used for light microscopy was as described by Geoffrey [23]. Paraffin sections of 5-μm thickness were cut by a rotatory microtome, mounted on clean slides and stained with haematoxylin and eosin for histopathological examination. Photomicrography of stained tissue slides were obtained using Lx 400 Labomed Trinocular Digital Microscope (Serial No: 150546516; Labo America Inc., Fremont, CA, USA) suite.

2.6 Epididymal sperm count, motility and morphology

The seminal fluid collected from the epididymis was utilized to evaluate the sperm count, sperm motility and sperm morphology across the study groups. The epididymal fluid ratio of 1:20 was prepared by adding 0.1 mL of fluid to 1.9 mL of water, and the sperm were counted in a Neubauer's counting chamber. The number of spermatozoa in two large squares is counted using the 10× objective. The epididymal sperm analysis protocol described in the manual of basic semen analysis was used in this study [24]. Sperm motility is investigated using a well-mixed drop of liquefied semen on a slide, with the 40× objective lens used to observe and count. A total of 100 spermatozoa were counted, with the number of motile ones recorded in percentage. The percentage of motile sperms was defined as the number of motile sperms divided by the total number of counted sperms. Sperm morphology was examined using a thin smear of liquefied well-mixed semen on a slide. The smear was fixed with a 1:2 10% neutral buffered formalin and counterstained with dilute Loeffle's methylene blue for 2 min washed off with water, drained and air-dried. The smear is examined for normal and abnormal spermatozoa using the 40× objective and the 100× objective to confirm abnormalities under the light microscope. Abnormal morphology was defined as spermatozoa having one or more of the following features: rudimentary tail, round head and detached head, expressed as a percentage of morphologically normal sperm.

2.7 Hormonal assay

The serum samples were analyzed for testosterone levels using the AccuBind enzyme-linked immunosorbent assay (ELISA) microwells for testosterone, purchased from Monobind Inc. Lake Forest, California, USA. The protocols used were as described in the Testosterone Test System with product code 3725-300 [25].

2.8 Serum kisspeptin determination

The serum kisspeptin levels were determined using MELSIN Kisspeptin ELISA kit (EKHU-2171; Changchun, China) with an assay range of 0–16 ng/mL, 0.1 ng/mL sensitivity, intra-assay coefficients of variability (CV) of <10%, and inter-assay CV of <15%. The stored plasma was thawed, and all sample reagents (20× wash solution—diluted with distilled or deionized water 1:20) were prepared before starting the assay procedure. The standards and samples were added in duplicate

to the ELISA plates. The standard and testing sample wells were set, and 50 μL and 10 μL were added to the standard wells, respectively. 10 μL sample diluent was added to the testing sample well, and 100 μL of Horseradish peroxidase (HRP)-conjugate reagent. The wells were covered with an adhesive strip, incubated for 60 min at 37 °C, aspirated, and repeatedly washed five times using a wash solution (400 μL). After the last wash, the remaining wash solution was removed by aspiration or decanting. The plates were inverted and blotted against clean paper towels. Chromogen solution A (50 μL) and Chromogen solution B (50 μL) were added to each well, mixed, and incubated for 15 min at 37 °C. The plates were protected from light. 50 μL stop solution was added to each well with a gentle tap to the plate for thorough mixing before reading the Optical density (OD) at 450 nm using a microtiter plate reader within 15 min. The standard curve was used to determine the amount of kisspeptin in the samples. Plotting the average OD (450 nm) attained for each of the standard concentrations against the corresponding concentrations on the horizontal (x) axis resulted in the creation of the standard curve. The concentration of the samples was determined by locating the OD value on the y-axis and extending a horizontal line to the standard curve to find a point of intersection.

2.9 Serum oxidant status

Malondialdehyde (MDA), Glutathione (GSH) and Catalase (CAT) levels were quantified in the serum to determine the oxidant status using the protocol described in a previous study [26].

2.10 Statistical analysis

Data was analyzed using the International Business Machines (IBM) statistical package for social sciences (SPSS) for Windows, version 23 (IBM Corporation, Armonk, NY, USA). Analysis of variance (ANOVA) and paired sample *t*-test statistics were employed to analyze the test parameters evaluated. All the results obtained were expressed as mean value ± standard error of mean and values were considered significant at $p < 0.05$.

3. Results

3.1 The effect of tramadol and vitamin D3 on the body weight

There was no significant difference ($p > 0.05$) in the pre- and post-administration body weights in all the experimental groups (Table 1).

3.2 The effect of vitamin D3 and tramadol on relative testicular weight

There was no significant difference ($p > 0.05$) in the relative testicular weight in all the experimental groups (Table 2).

TABLE 1. The body weight of rats before and after tramadol and vitamin D3 administration.

Groups	Body weight (g) (Mean ± SEM)	*p*-value
A		
Pre-administration body weight	135.48 ± 1.31	0.085
Post-administration body weight	156.30 ± 9.43	
B		
Pre-administration body weight	139.82 ± 6.41	0.051
Post-administration body weight	168.42 ± 5.96	
C		
Pre-administration body weight	149.44 ± 3.40	0.058
Post-administration body weight	162.35 ± 5.20	

SEM: standard error of mean; data was analyzed using a paired sample t-test and values were considered significant at $p < 0.05$ compared to the control (group A).

TABLE 2. The relative testicular weight in rats following the administration of tramadol and vitamin D3.

Groups	Relative testicular weight (Mean ± SEM)	*p*-value
A	1.61 ± 0.10	
B	1.20 ± 0.14	0.030
C	1.55 ± 0.09	0.709

Data was analyzed using paired sample t-test and values were considered significant at $p < 0.05$ compared to control (group A). SEM: standard error of mean.

3.3 The effect of tramadol and vitamin D3 on kisspeptin levels

There was no significant difference ($p > 0.05$) in the serum kisspeptin levels in all the experimental groups (Table 3).

TABLE 3. The effect of tramadol and vitamin D3 on kisspeptin levels.

Groups	Kisspeptin levels (ng/mL) (Mean ± SEM)	*p*-value
A	3.08 ± 0.09	
B	2.96 ± 0.06	0.250
C	3.01 ± 0.05	0.422

Data was analyzed using one-way ANOVA and values were considered significant at $p < 0.05$ compared to control (group A). SEM: standard error of mean.

3.4 The effect of vitamin D3 and tramadol on sperm parameters

The result shows no significant difference in actively motile sperm, non-motile sperm, total sperm count, normal and abnormal sperm cells in all the experimental groups (Table 4).

TABLE 4. The effect of vitamin D3 and tramadol on sperm parameters.

Semen Parameters Groups	Test value	*p*-value
Actively Motile (%)		
A	88.33 ± 1.67	
B	84.00 ± 5.03	0.562
C	81.33 ± 6.84	0.360
Non-Motile (%)		
A	11.67 ± 1.67	
B	16.00 ± 5.03	0.562
C	18.67 ± 6.84	0.360
Total Sperm Count ($\times 10^6$/mL)		
A	77.50 ± 3.69	
B	53.57 ± 15.30	0.116
C	94.50 ± 2.55	0.240
Normal Sperm cells (%)		
A	85.00 ± 2.89	
B	78.33 ± 1.67	0.071
C	86.67 ± 1.67	0.604
Abnormal sperm cells (%)		
A	15.00 ± 2.89	
B	21.67 ± 1.67	0.071
C	13.33 ± 1.67	0.604

Data was analyzed using One-way ANOVA and post-hoc LSD test, and values were considered significant at $p < 0.05$ compared to control (group A). SEM: standard error of mean.

3.5 The effect of vitamin D3 and tramadol on testosterone level

The study found no significant difference in the serum testosterone levels across all the study groups (Table 5).

TABLE 5. The effect of vitamin D3 and tramadol on testosterone level.

Groups	Testosterone (ng/mL) Mean ± SEM	*p*-value
A	0.820 ± 0.512	
B	0.195 ± 0.094	0.241
C	0.365 ± 0.082	0.367

Data was analyzed using One-way ANOVA and post-hoc LSD test, and values were considered significant at $p < 0.05$ compared to control (group A). SEM: standard error of mean.

3.6 The effect of tramadol and vitamin D3 on the oxidative status of the testis

The result showed non-significant differences in the serum oxidative status markers (CAT, GSH and MDA) in all the experimental groups (Table 6).

TABLE 6. The effect of tramadol and vitamin D3 on the oxidative status of the testis.

Variables Groups	Mean ± SD	*p*-value
CAT		
A	32.3400 ± 1.75362	
B	35.6950 ± 2.56680	0.419
C	30.7850 ± 5.38108	0.694
GSH		
A	46.5700 ± 10.09748	
B	37.8450 ± 0.79903	0.278
C	36.4800 ± 5.30330	0.224
MDA		
A	3.1600 ± 1.04652	
B	2.7600 ± 0.16971	0.571
C	2.9150 ± 0.26163	0.724

The table was analyzed using one-way ANOVA and values were considered significant at $p < 0.05$ compared to control (group A). SD: Standard Deviation; CAT: Catalase; GSH: Glutathione; MDA: Malondialdehyde.

3.7 Histological results

In the control group (group A), the testicular histoarchitecture appeared normal (Fig. 1).

The tramadol-treated group (group B) exhibited notable alterations in testicular histoarchitecture. The photomicrograph (Fig. 2) revealed that while there was still a presence of dividing spermatogonia within each seminiferous tubule and interstitial cells in the adjoining spaces, there was a marked loss and degradation of spermatozoa.

The histological examination of the testis in the group treated with both tramadol and vitamin D3 (group C) showed a histoarchitecture like that of group B. The photomicrograph (Fig. 3) demonstrated a pool of dividing spermatogonia within each seminiferous tubule and interstitial cells arranged in the spaces between tubules. However, there was a significant loss and degradation of spermatozoa, like the tramadol-only group.

4. Discussion

This preliminary study evaluated the effect of the coadministration of vitamin D3 and tramadol on the serum kisspeptin levels, testicular histology, semen parameters, testosterone levels and oxidative stress in rats to delineate the possible protective effect of vitamin D3 on tramadol-induced reproductive damage and the associated mechanism of action. The results of this study indicated that there were no significant differences in body weights or relative testicular weights be-

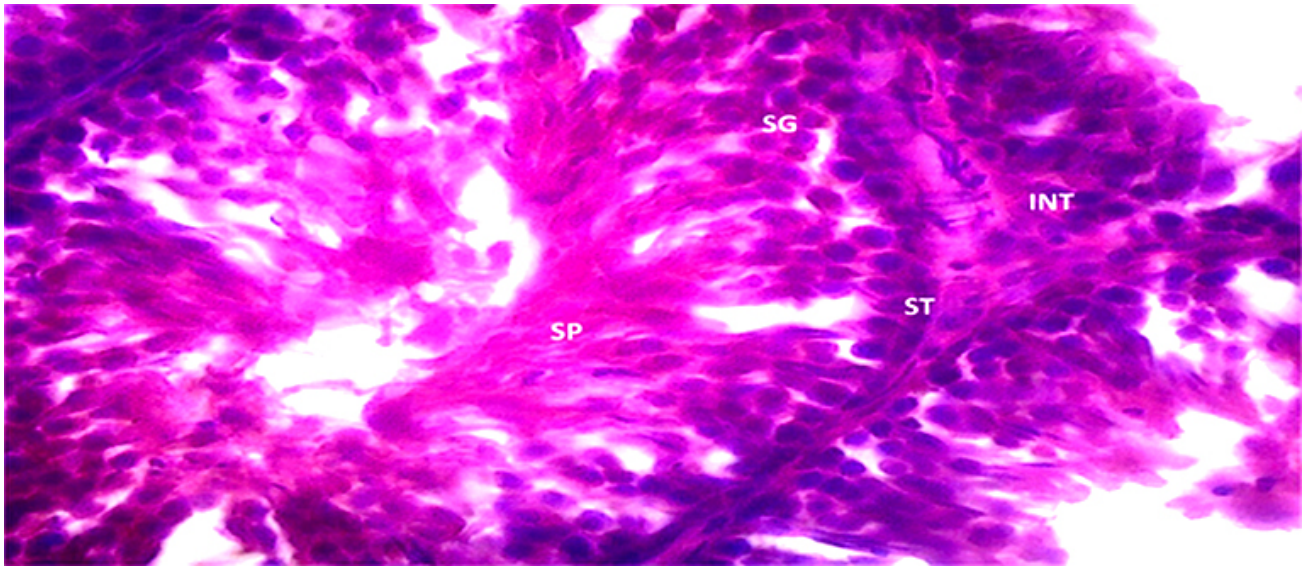

FIGURE 1. Histopathological image of group A testicular tissue treated with distilled water only (H&E), ×400. The testis photomicrograph illustrates a normal and well-organized histoarchitecture. Within each seminiferous tubule, there is a visible pool of dividing spermatogonia (SG), which are the germ cells responsible for initiating spermatogenesis. These cells are situated near the basal membrane of the tubule and represent the earliest stage in the development of sperm. As spermatogenesis progresses, these spermatogonia mature and move towards the lumen of the tubule, ultimately differentiating into fully developed spermatozoa (SP), which are present in the central lumen of the tubule. In the spaces between the seminiferous tubules, the interstitial cells (INT), or Leydig cells, are clearly visible. These cells are strategically located in the interstitial tissue and play a crucial role in producing testosterone. The overall structure depicted in this photomicrograph reflects a healthy testicular environment, where the processes of germ cell development and hormone production are functioning normally. The arrangement of the spermatogonia, maturing spermatozoa, and interstitial cells suggests that the testicular tissue is structurally intact and capable of supporting regular sperm production. ST: seminiferous tubule.

tween the experimental groups. The stability of these parameters suggests that tramadol at the tested dose and duration did not induce systemic toxicity, organ-specific damage or impacts severe enough to cause noticeable weight alterations. Mohamed and Mahmoud's study [27], which administered 30 and 60 mg/kg tramadol for eight weeks without causing any significant changes in body weight, is consistent with these findings. Kisspeptin, a critical regulator of the hypothalamic-pituitary-gonadal (HPG) axis, plays a crucial role in reproductive function and pubertal development [15]. Previous research has shown that various opioids can disrupt the HPG axis, potentially influencing kisspeptin expression and release [28]. However, our findings show that tramadol, an atypical opioid, does not significantly alter kisspeptin levels. It also suggests that tramadol and vitamin D3 administration did not influence kisspeptin secretion in these rats.

Semen analysis revealed no significant differences in actively motile sperm, non-motile sperm, total sperm count and normal or abnormal sperm cells across the groups upon exposure to tramadol alone or in combination with vitamin D3. This is somewhat unexpected, given previous studies that reported tramadol-induced sperm abnormalities. Koohsari and colleagues [29] found that rats exposed to tramadol had sperm dysfunctions and a significant decrease in sperm count, motility and morphology. Azari and colleagues [30] found a significant link between chronic tramadol administration and impaired quality of sperm parameters, including a decrease in sperm count, motility and vitality in mice. It is important to note that the effect of tramadol and vitamin D3 co-administration on semen parameters seen in this present study is concomitant with the observed effect on the serum testosterone levels, which also showed no significant effect across all groups, even though previous research has shown that tramadol or its opioid equivalents can significantly reduce testosterone levels [31–37]. Previous studies and this current study may differ in their effects due to the relatively short study duration or the administered tramadol dose. Future studies with extended durations and varied dosing might provide more insight.

In this study, oxidative stress and lipid peroxidation markers did not show any significant changes following rat exposure to tramadol and vitamin D3. This finding agrees with the study by Koohsari and colleagues [29], whose assessment of reactive oxygen species and lipid peroxidation was not significant following the administration of 25 mg/kg tramadol to rats for three weeks. However, in same study, exposure to higher doses (50 mg/kg and 70 mg/kg) of tramadol caused a significant alteration in the oxidative stress status. It is safe to infer from the findings of this present study that the dosage and duration of tramadol administration were insufficient to produce measurable changes in the oxidative stress and lipid peroxidation parameters. It is important that future studies consider more spectrum of oxidative stress indices to understand the relationship between the tramadol administration and oxidative stress induction to enable more therapeutic pathways for tramadol-induced damage.

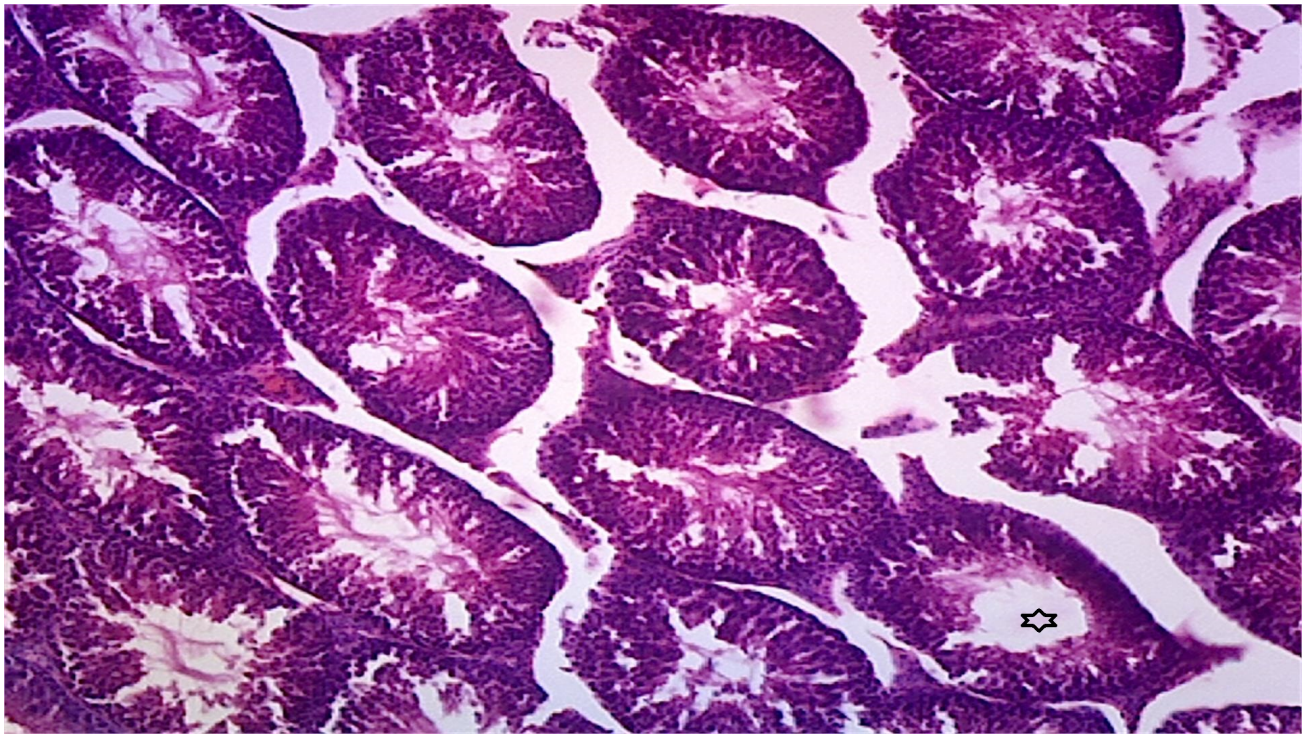

FIGURE 2. Histopathological image of group B testicular tissue treated with 20 mg/kg tramadol only (H&E), ×100. The testis photomicrograph reveals the intricate histoarchitecture of the seminiferous tubules, which are the sites of sperm production. Within each seminiferous tubule, a pool of dividing spermatogonia can be observed near the basement membrane, marking the initial stage of spermatogenesis. These germ cells progressively mature as they move towards the lumen of the tubule, where they normally develop into fully formed spermatozoa. The interstitial spaces between the seminiferous tubules are populated with Leydig cells, which are crucial for the production of testosterone. These cells are distributed in the connective tissue surrounding the tubules, forming a supportive framework for the seminiferous epithelium. However, the photomicrograph indicates a significant pathological finding: a marked loss or degradation of spermatozoa within the lumen of the seminiferous tubules, as highlighted by the starred area. This reduction in the number of mature sperm cells suggests a disruption in the normal process of spermatogenesis. The observed degradation may be indicative of testicular damage.

Histological examination provided more definitive evidence of tramadol's impact. The tramadol-treated group exhibited notable testicular damage, characterized by the degradation of spermatozoa. This finding is consistent with prior studies showing tramadol's deleterious effects on testicular structure [8, 30, 35, 38]. In this study, the co-administration of vitamin D3 did not significantly ameliorate the damage caused by tramadol. The lack of significant protective effects from vitamin D3 in this study contrasts with some reports that have highlighted its beneficial role in supporting cellular growth and protecting against various forms of testicular damage [20, 39–41]. These differences are also attributable to variations in study design, including drug doses and duration of treatment. The current findings do not fully elucidate the molecular mechanisms underlying tramadol-induced testicular damage and the potential protective role of vitamin D3. Future studies should consider longer treatment durations, different dosages of tramadol and vitamin D3, additional reproductive hormones, and stereological studies on the seminiferous tubules to understand the dose-dependent relationships and synergistic effects amongst varying reproductive parameters. Understanding these mechanisms may provide insights into more effective strategies for mitigating the adverse effects of tramadol on male reproduction.

5. Conclusions

The acute exposure of rats to tramadol has no deleterious effect on the sperm parameters, serum kisspeptin levels, serum testosterone levels or oxidative stress markers. However, the coadministration of vitamin D3 does not attenuate the marked degeneration of spermatozoa in the testicular tissue.

AVAILABILITY OF DATA AND MATERIALS

The data presented in this study are available on reasonable request from the corresponding author.

AUTHOR CONTRIBUTIONS

IAO and SIM—designed the research study. IAO, IGC, ECA and IMA—performed the research. IAO and IMA—provided help and advice on protocols. CEO and IAO—analyzed the data. CEO, IGC, ECA and IAO—wrote the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

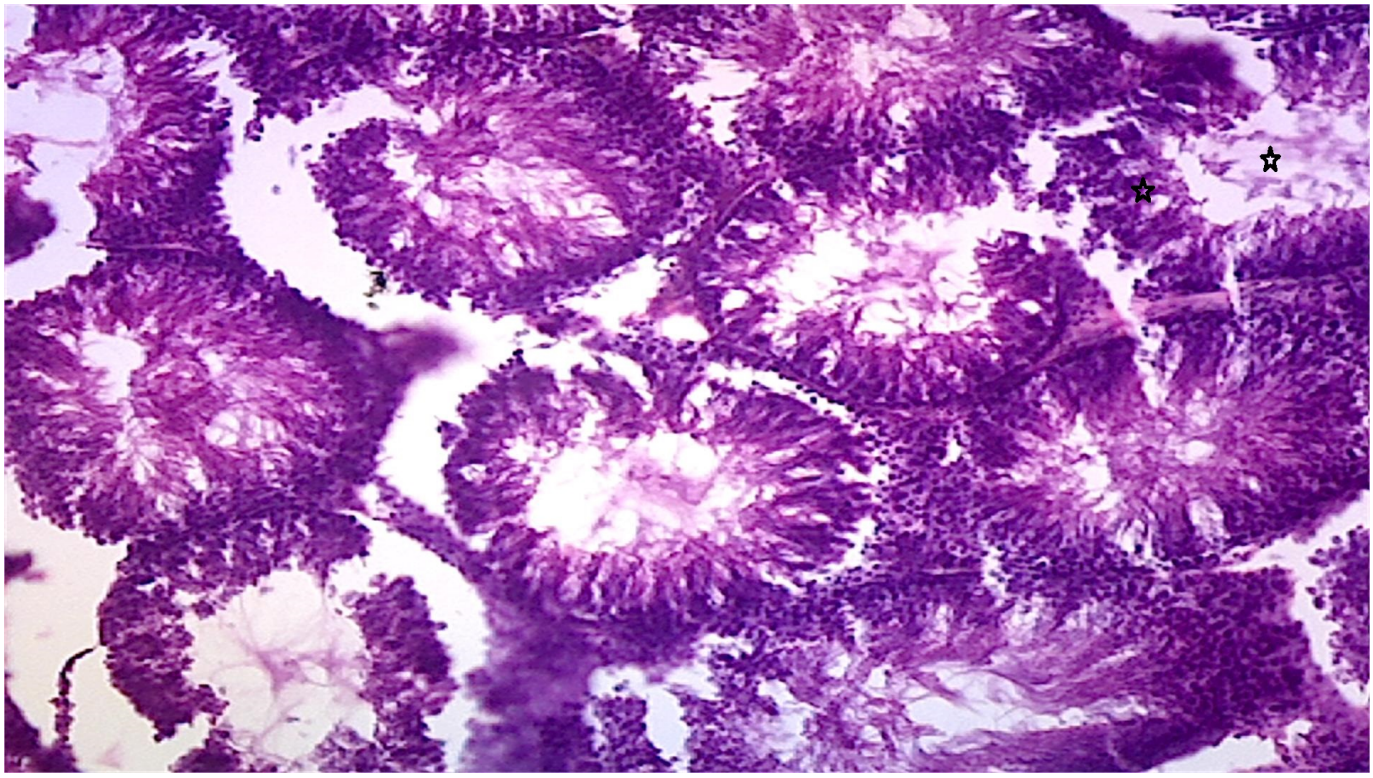

FIGURE 3. Histopathological image of group C testicular tissue treated with 20 mg/kg tramadol + 25 μg vitamin D3 (H&E), $\times$100. The testis photomicrograph illustrates the detailed histoarchitecture of the seminiferous tubules and surrounding interstitial tissue. Each seminiferous tubule displays a layered arrangement of germ cells at various stages of spermatogenesis, beginning with spermatogonia near the basement membrane and progressing towards the lumen, where mature spermatozoa are typically found. The seminiferous tubules are lined with Sertoli cells that support the development of these germ cells. In the interstitial spaces between the tubules, clusters of Leydig cells are observed, responsible for testosterone production. However, a marked degradation of spermatozoa is evident in the lumen of the seminiferous tubules, as highlighted by the starred area. This degradation suggests a disruption in the process of spermatogenesis, possibly indicative of testicular damage or dysfunction. The loss of spermatozoa in tubules points to a reduction in the number of mature sperm cells. The interstitial cells, though still present, appear to be less densely arranged.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study received ethical approval from the Research Ethics Committee of the Faculty of Basic Medical Sciences, Nnamdi Azikiwe University Nnewi Campus, with approval number: NAU/CHS/NC/FBMS/670. The experimental procedures complied with ARRIVE guidelines and the National Institutes of Health Guide for the Care and Use of Laboratory Animals. No consent was required for this study.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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