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Research Article

Effect of Fentanyl Administration on Sperm Parameters and Testicular Histoarchitecture in Male Wistar Rats

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ABSTRACT

Fentanyl, a synthetic opioid, is used pharmaceutically for pain management, even as concerns has consistently been raised about its growing misuse, and the consequences on delicate body systems such as the male reproductive system. Even though opioids are known to affect the endocrine system, the evidence of fentanyl's direct influence on sperm quality and testicular histology have been under-explored. This study aimed to evaluate the effect of fentanyl administration on sperm parameters, serum testosterone levels, and testicular histology in adult male Wistar rats. Twenty-five (25) adult male Wistar rats were randomly divided into five groups (n=5). The control group received 5 mL/kg distilled water, while groups 2–5 received intramuscular fentanyl at doses of 1 µg/kg, 1.5 µg/kg, 2 µg/kg, and 2.5 µg/kg, respectively, for 21 d. After treatment, body weight, serum testosterone concentration, and sperm indices were assessed, and testicular tissues were histologically examined using hematoxylin and eosin staining. All groups gained weight over the course of the study, but treated groups showed a non-significantly smaller weight gain than control, with the smallest weight gain recorded in group 5 (57.32±8.03 g) compared to the control (86.00±24.02 g) ($p>0.05$). Testosterone levels decreased slightly in fentanyl groups (0.98±0.15 ng/mL to 1.30±0.10 ng/mL) relative to control (2.93±0.92 ng/mL). Sperm count increased in treated rats, peaking in group 4 (51.13±17.54) compared to control (26.75±22.45), though not significantly. Testicular histology revealed intact seminiferous tubules, normal spermatogenic architecture, and preserved Leydig cells across all groups. Fentanyl administration for 21 d induced mild, non-significant alterations in body weight, testosterone levels, and sperm parameters, without histological evidence of testicular damage. The results of this research show that intramuscular fentanyl at the doses administered in this study could influence latent reproductive effects. Biomarker based approaches could be a better option in reproductive toxicology when carrying out preclinical drug safety analysis and reproductive risk evaluation.

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INTRODUCTION

The testes are vital components of the male reproductive system whose main functions include the production of spermatozoa and the secretion of androgens, especially testosterone, which is crucial to fertility in males (Speiser *et al*., 2018). The testes are endowed with very important endocrine functions that are essential to fertility in the males. However, exposure to certain pharmacological agents and environmental toxins can impair testicular function, leading to conditions such as infertility, hypogonadism, and testicular atrophy (Hauger *et al.*, 2020).

Anabolic-androgenic steroids, including testosterone derivatives such as nandrolone and stanozolol, are widely misused for performance enhancement among athletes but have been shown to exert deleterious effects on testicular structure and function (Krasowski *et al.*, 2014; Kanou *et al.*, 2020). Similarly, testicular dysfunctions resulting in altered spermatogenesis and even hormonal imbalance have been linked to opioids like heroin, fentanyl, and morphine in the studies of Coluzzi *et al*. (2015). While some report indicates that fentanyl administration may have some protective effect against ischemia-reperfusion injury (Cengiz *et al.*, 2021), the consequence of its exposure on reproductive virility remains poorly understood and warrants further investigation.

Fentanyl has been reported to be about 30 – 50 times more effective than heroin, and approximately 100 times more active than morphine as a synthetic opioid analgesic, with a rapid onset of activity, and short duration of action (James *et al.*, 2018). It is used clinically for severe pain management especially in cancer patients, as a post-operative analgesic, and a sedative agent (Ramos *et al.*, 2022), exerting its analgesic effects through the μ-opioid receptors in the brain and spinal cord, to modulate severe pain (Hemmings *et al.*, 2018). When administered, it induces sedation and drowsiness, and if at higher doses, it generates δ-wave patterns on electroencephalogram, just like those seen during natural sleep (Hemmings *et al.*, 2018). Owing to its high potency and lipophilicity, fentanyl acts rapidly depending on the route of administration, and even minimal overdoses can be fatal (Lukić *et al.*, 2021).

Even though its therapeutic uses are well-known, concerns have been raised about fentanyl's misuse have been raised and its resultant effect on the reproductive system given its endocrine-disrupting effects, even though evidence of its effect on the male reproductive system have been limited.

This study thus aims to evaluate the impact of fentanyl treatment on sperm parameters and testicular histoarchitecture in male Wistar rats, thereby contributing to a better understanding of its possible reproductive risks and guiding safer clinical and non-clinical opioid use.

MATERIALS AND METHODS

Ethical Clearance

The National Health Research Ethics Committee's (NHREC) recommendations for the care and use of laboratory animals were strictly adopted in this study. Furthermore, the Ethical Committee of the Department of Anatomy, Faculty of Basic Medical Sciences, Baze University after reviewing the research protocol approved the research and issued a reference number BU/URES/ANA/1000 on approval.

Procurement and Housing of Research Animals

A total of twenty five (25) male Wistar rats were procured from, and housed in the Animal House of the College of Medicine and Health Sciences, Baze University, Abuja in five well-ventilated plastic cages measuring 20cm x 30cm. The rats were fed *ad libitum* with vital feed and water, and kept at 25°C through a 12 h light and dark alternate cycles throughout the period of the research, which included a 14 d acclimatization period, and a 21 d treatment period. The animals were weighed before and after acclimatization, and also before and after the administration using an electric weighing balance.

Procurement and Preparation of Experimental Drug

The drug fentanyl was purchased from Ruvi pharmacy, a licensed pharmaceutical outfit.

The drug was stored at a temperature range of 22^0C – 25^0C in a temperature-controlled refrigerator at the Animal House of the College of Medicine and Health Sciences, Baze University, Abuja.

Experimental Design

The male Wistar rats were divided into five (5) groups randomly, consisting of five rats each, and fed with rat feed (vital feed) that was purchased from a feed store in Abuja. The rats were administered fentanyl in research grade with the dosage as follows:

Group 1 (Control): 5ml/kg distilled water for 21 d

Group 2: 1mcg/kg of fentanyl for 21 d intramuscularly

Group 3: 1.5mcg/kg of fentanyl for 21 d intramuscularly

Group 4: 2mcg/kg of fentanyl for 21 d intramuscularly

Group 5: 2.5mcg/kg of fentanyl for 21 d intramuscularly

Animal Sacrifice

After a 21 d treatment period, all 25 Wistar rats were humanely sacrificed by chloroform anaesthesia after prior overnight fast. Blood samples were drawn in sterile bottles for biochemical analysis, and the organs (testis and epididymis) were excised and fixed in 10% formaldehyde for histological tissue processing.

Measurement of Testosterone Concentration

Serum testosterone levels were measured through the Enzyme Immunoassay (EIA) technique using ELISA kit (DRG Testosterone ELISA; EIA-1559), that is based on the principle of competitive binding (Tietz, 1995). Incubation of Goat anti-rabbit IgG-coated microplate wells were done with testosterone (TT) standards, controls, samples (serum and testicular homogenate supernatants), TT-horseradish peroxidase conjugate, and rabbit anti-TT reagent at 37°C for 90 min. After incubation, unbound conjugate was removed by washing, and tetramethylbenzidine substrate was added to develop color. The reaction was terminated with 1N hydrochloric acid, and absorbance was measured at 450 nm using a spectrophotometer. Testosterone concentrations were calculated from a standard curve generated by plotting standard concentrations against absorbance values.

Sperm Analysis

Sperm parameters were evaluated following a modified method of Seed *et al*., (1996). The cauda epididymis was minced in 5 mL of physiological saline. Before the incubation that was done at room temperature for 2 min, the solution was agitated gently for 10 min. The supernatant was diluted (1:100) in a solution containing 5 g sodium bicarbonate and 1 mL of 35% formalin. Total sperm count was determined using an improved Neubauer hemocytometer. A 10 µL aliquot of the diluted suspension was loaded into each chamber, allowed to settle, and examined under a light microscope. Spermatozoa were counted in five 16-celled squares, and the concentration was expressed as $(X\times10^6)$/mL, where X represents the mean sperm count.

Sperm motility was assessed microscopically at ×40 magnification using samples from the original dilution. For morphological evaluation, spermatozoa were fixed in 10% neutral buffered formalin (Sigma-Aldrich, Oakville, ON, Canada) at a 1:20 dilution.

A total of 500 spermatozoa per sample were examined under phase-contrast microscopy, and morphological abnormalities were recorded. The percentage of morphologically normal spermatozoa was calculated, and representative micrographs were taken for reference.

Histological Tissue Processing and Examination

The fixed testicular tissues were passed through 70 – 95% grades of alcohol and absolute ethanol, and then cleared through xylene and passed through molten paraffin wax for embedding.

Ultrathin tissue sections were obtained from blocks of paraffin wax which were frozen at 5^0C for 15 min using the rotary microtome set at 3 µm. The sections were floated in water bathe at 55^0C, mounted on glass slides and dried on a hot plate for 40 min for adhesion.

The slides were dewaxed in xylene, and then hydrated in descending alcohol grades. Harris hematoxylin was used to stain for 5 min after which they were run through 1%

acid alcohol, washed rinsed under a running tap water, and counterstained in 1% eosin. The sections were again dehydrated through ascending grades of alcohol, cleared in xylene, and mounted with dibutylphthalate propylene xylene.

Stained slides were viewed under a compound light microscope to examine the testicular histology.

Statistical Analysis

Data analysis was done using IBM SPSS version 23.0. Mean and Standard Deviations (SD) were computed for all groups, and inter-group comparison as done by One-Way Analysis of Variance (ANOVA) and LSD post-hoc tests. Statistical significance was placed at $p<0.05$.

RESULTS

Body Weight Measurements across Groups

The results in Table 1 show that every group, including all fentanyl-treated groups, had a higher final body weight than initial body weight, indicating that weight gain occurred throughout the study in all groups. However, the magnitude of weight gain was smaller in the treated groups than in the control group (86.00±24.02%), with group 5 (57.32±8.03%) showing the smallest weight gain, followed by group 4 (63.13±29.41%), group 2 (73.38±24.38%), and group 3 (81.38±26.16%). For the initial body weight, group 2 showed a significantly higher initial body weight compared to the control; since animals were randomly assigned to groups, this baseline imbalance is acknowledged as a potential confound. For the mean body weight changes, no group showed a statistically significant difference when compared to the control group ($p>0.05$). These findings suggest that fentanyl may be associated with a smaller weight gain in male Wistar rats rather than actual weight loss, although this was not statistically significant.

Table 1: Body Weight Changes across Groups

GROUP (N)	INITIAL WEIGHT (g)	FINAL WEIGHT (g)	WEIGHT CHANGE (g)
Group 1 (Control)	110.60±14.38	196.60±13.26	86.00±24.02
Group 2 (1mcg/kg FTL)	132.25±15.76*	205.63±36.22	73.38±24.38
Group 3 (1.5mcg/kg FTL)	115.75±7.37	197.13±33.11	81.38±26.16
Group 4 (2mcg/kg FTL)	118.50±7.59	181.63±34.35	63.13±29.41
Group 5 (2.5mcg/kg FTL)	116.43±13.38	173.75±12.22	57.32±8.03

Values as MEAN±SD; N = 5; FTL = Fentanyl; *$P<0.05$ to Control

Testosterone Levels across Groups

From the data in Table 2, group 2 - 5 showed little decrease in mean testosterone levels (ng/ml) against the control group (2.93±0.92 ng/ml) with group 2 showing the highest decrease (0.98±0.15 ng/ml) followed by group 3 (1.17±0.06 ng/ml), group 5 (1.27±0.15 ng/ml) and group 4 (1.30±0.10 ng/ml) in that order. The mean testosterone levels in groups 2 and 3 showed statistically significant decreases ($p<0.05$) as against the control group. These findings suggest that fentanyl, especially at 1mcg and 1.5mcg respectively showed a decreasing effect on testosterone production.

Table 2: Mean Testosterone across Groups

GROUP (N)	TESTOSTERONE (ng/mL)
Group 1 (Control)	2.93±0.92
Group 2 (1mcg/kg FTL)	0.98±0.15*
Group 3 (1.5mcg/kg FTL)	1.17±0.06*
Group 4 (2mcg/kg FTL)	1.30±0.10
Group 5 (2.5mcg/kg FTL)	1.27±0.15

Values as MEAN±SD; N = 5; FTL = Fentanyl; *P<0.05 to Control

Sperm Parameters: Sperm Count, Motility, Morphology, and Progressivity

Table 3 shows the variations in sperm parameters. Increases in the mean sperm count across groups were observed in comparison to the control group, with group 4 (51.13±17.54) showing the highest increase followed by group 2 (49.65±7.23), group 3 (35.47±16.14) and group 5 (32.87±24.34) as against the control group (26.75±22.45). Groups 2 and 4 showed statistically significant increases in sperm count ($p<0.05$) compared to the control group (group 1). For sperm motility, groups 2 and 3 showed a decrease in mean percentage of sperm motility compared to the control group (41.83±28.17%), with group 2 showing a statistically significant decrease (25.78±20.66%) compared to the control, followed by group 3 (36.00±27.55%). Group 5 (1.27±0.15 ng/ml) and group 4 (1.30±0.10 ng/ml) showed increase in mean percentage of sperm motility. Groups 2 - 4 showed increases in the mean sperm morphology compared to the control group (51.75±33.38%) with group 2 showing the highest increase (85.55±5.39%), followed by group 4 (74.20±14.76%), which were statistically significant to the control. Group 5 showed a slight decrease in mean when compared to the control group (48.47±13.05%). For sperm progressivity, no statistically significant difference in mean was observed across the experimental groups compared to the control (Table 3).

Table 3: Mean Sperm Parameters across Groups

GROUP (N)	COUNT (x10^6/mL)	MOTILITY (%)	MORPHOLOGY (%)	PROGRESSIVITY (Scale 1 - 4)
Group 1 (Control)	26.75±22.45	41.83±28.17	51.75±33.38	1.50±0.58
Group 2 (1mcg/kg FTL)	49.65±7.23*	25.78±20.66*	85.55±5.39*	1.00±0.58
Group 3 (1.5mcg/kg FTL)	35.47±16.14	36.00±27.55	60.20±16.10	2.00±0.82
Group 4 (2mcg/kg FTL)	51.13±17.54*	51.13±17.54	74.20±14.76*	1.75±0.50
Group 5 (2.5mcg/kg FTL)	32.87±24.34	46.80±36.41	48.47±13.05	1.50±0.58

Values as MEAN±SD; N = 5; FTL = Fentanyl; *P<0.05 to the Control

Histological Evaluation

The microscopic evaluation of testicular samples from Group 1 exhibited a well-preserved and structurally sound histological profile. The seminiferous tubules displayed an organized arrangement, with germ cells at different maturation phases systematically aligned. A substantial presence of spermatozoa was evident, oriented towards the tubule lumens, while the interstitial regions contained intact Leydig cells with no apparent abnormalities. Minimal retention of spermatids was observed, though this was deemed non-pathological. Groups 2 through 5 showed testicular morphology that mirrored that of Group 1. The seminiferous tubules in these groups retained their structural coherence, with no indications of atrophy, germ cell disarray, or degenerative transformations. The interstitial spaces were well-preserved, and Leydig cells maintained their normal morphology, showing no evidence of necrosis or irregularities. Microscopic examination further confirmed that the seminiferous tubules across all groups housed developing spermatogenic cells with well-defined nuclear membranes. No signs of karyorrhexis, nuclear fragmentation, or other cellular deterioration were detected. Spermatogonia displayed typical nuclear morphology, and the luminal spaces of the seminiferous tubules contained mature spermatozoa without traces of cellular debris or degenerative changes. The basement membranes encasing the seminiferous tubules remained unaltered, without any signs of thickening or hyalinization. The histological features described above were consistently observed across all five animals examined per group, and the photomicrographs presented below (Plates 1–5) are representative of each group.

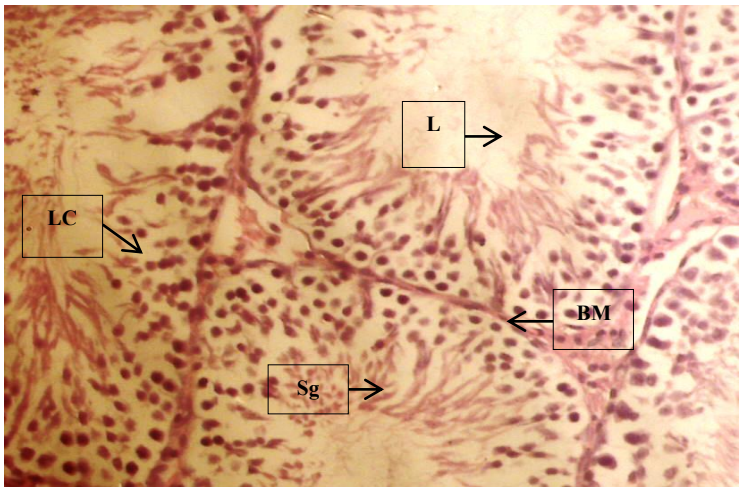

Plate 1: Photomicrograph of the Testis from Group 1 showing Normal Histological Features with intact Lumen (L), Basement Membrane (BM), Spermatogonia (Sg), & Leydig Cells (LC) (H&E x10)

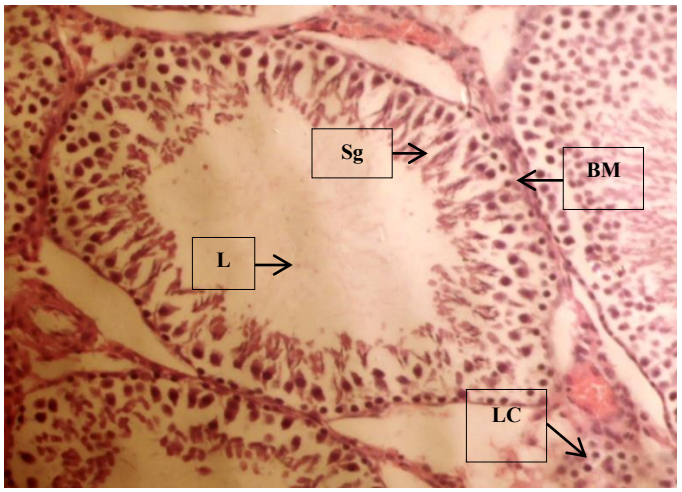

Plate 2: Photomicrograph of the Testis from Group 2 showing Histological Features with intact Lumen (L), Basement Membrane (BM), Spermatogonia (Sg), & Leydig Cells (LC) (H&E x10)

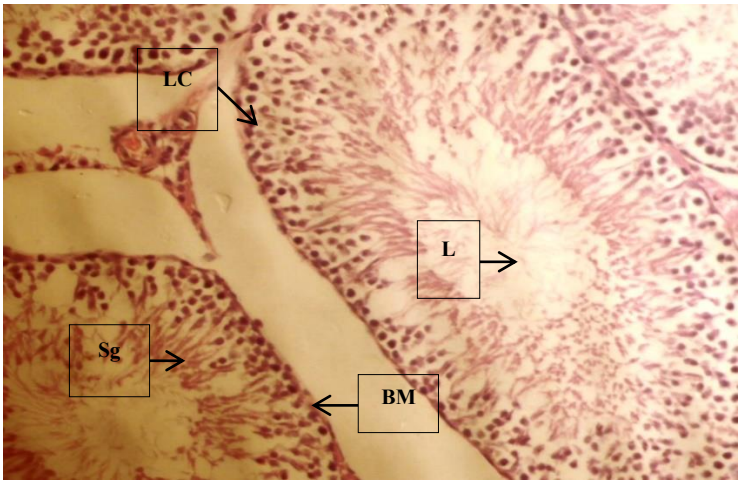

Plate 3: Photomicrograph of the Testis from Group 3 showing Normal Histological Features with intact Lumen (L), Basement Membrane (BM), Spermatogonia (Sg), & Leydig Cells (LC) (H&E x10)

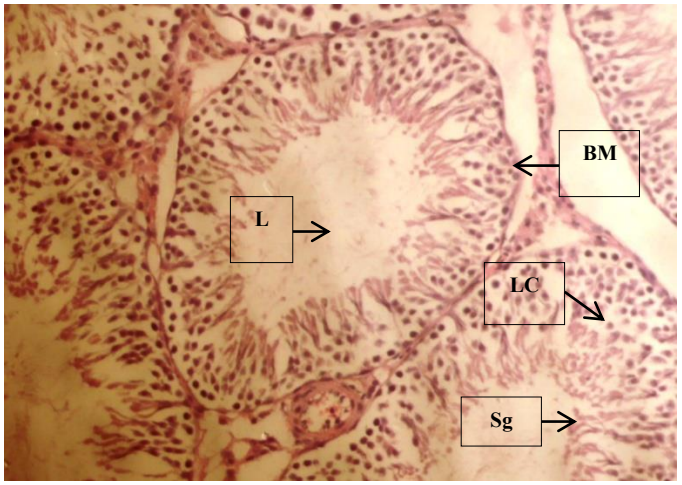

Plate 4: Photomicrograph of the Testis from Group 4 showing Normal Histological Features with intact Lumen (L), Basement Membrane (BM), Spermatogonia (Sg), & Leydig Cells (LC) (H&E x10)

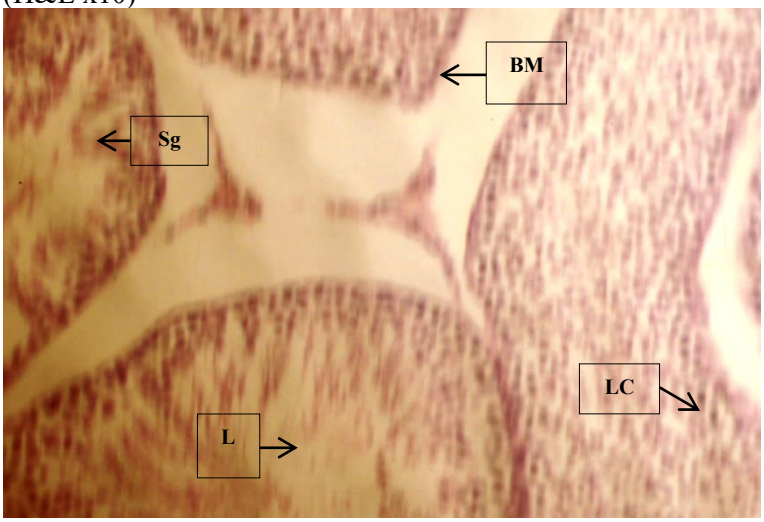

Plate 5: Photomicrograph of the Testis from Group 5 showing Normal Histological Features with intact Lumen (L), Basement Membrane (BM), Spermatogonia (Sg), & Leydig Cells (LC) (H&E x10)

DISCUSSION

Fentanyl administration in this study resulted in observable changes in parameters such as body weight, testosterone levels and sperm parameters, with preserved testicular histology. All groups, including the fentanyl-treated groups, gained weight over the course of the study; however, the fentanyl-treated groups showed a smaller weight gain relative to the control group, suggesting that the drug may have a mild weight gain-limiting effect rather than causing actual weight loss, though the differences were not statistically significant. It is also worth noting that Group 2 had a significantly higher initial body weight than the control before treatment began. Although animals were randomly assigned to groups, this baseline imbalance should be acknowledged as a potential confound, particularly as Group 2 also showed some of the most pronounced treatment-associated changes later in the study, including the largest testosterone decrease and the largest motility decrease; the extent to which these findings reflect a true treatment effect versus the baseline

difference cannot be fully disentangled with the present design. Other possible factors that could be responsible for the observed weight variation include nutrition, growth phase of the rats, environmental conditions, and physical stress. These findings agree with the results of Mitzelfelt (2010) that reported decreased food consumption and long-term body weight reduction after chronic fentanyl administration in aged rats; however, owing to differences in age and dosing regimens, direct comparison is limited. It remains possible that a longer treatment period, higher doses or a larger sample size may yield statistically significant weight differences; further, a smaller weight gain does not necessarily reflect direct reproductive toxicity but may signal general systemic effects of fentanyl.

All fentanyl-treated groups recorded lower mean testosterone levels than control, with Groups 2 and 3 showing statistically significantly lower testosterone levels compared to the control group. The observations reinforce the established mechanism of action of opioids on the hypothalamic-gonadal axis, inhibiting gonadotropin-releasing hormone (GnRH) and luteinising hormone (LH) release, thereby lowering testosterone production from Leydig cells (Elliot, 2012). Duca *et al.* (2019) also described opioid-induced hypogonadism, reduced androgen levels and impaired spermatogenesis in animal and human studies.

However, compared to other research for high-dose or prolonged opioid exposure which show more robust testosterone suppression (Drobnis & Nangia, 2017), the results suggest that the dosing regimen (relatively low µg/kg doses for 21 d) may not have been sufficient to elicit a strong endocrine disturbance.

Compared with the control group, sperm count increased across all treatment conditions. Sperm motility, however, demonstrated a biphasic trend: declining in Groups 2 and 3 relative to control, yet showing non-significant elevations in Groups 4 and 5. Normal sperm morphology increased in Groups 2–4 and marginally decreased in Group 5. Parallel to testosterone levels which exhibited maximum suppression at the lowest dosage (Group 2, 1 mcg/kg) followed by progressive recovery at higher doses (Groups 4 and 5) neither sperm count nor motility displayed a clear dose-response relationship. Compensatory feedback mechanisms within the spermatogenic pathway or phase-specific opioid sensitivity offer potential biological explanations; however, given the limited cohort size (n = 5/group) and absence of statistical significance, this non-monotonic pattern may simply reflect random inter-individual variance rather than a true pharmacological effect. As reviewed in Hamed *et al*. (2023), psychoactive drugs including opioids often impair sperm motility, morphology and promote oxidative-stress-mediated damage (Hamed *et al*., 2023).

A limitation of this study is the reliance on Fisher's LSD post-hoc test without adjustment for multiple comparisons across four treatment arms and six endpoint metrics. This analytical approach increases the risk of false positives (Type I error), meaning certain observed effects may reflect multiplicity rather than true biological efficacy. Future analyses incorporating adjusted post-hoc procedures (e.g., Tukey's HSD or Bonferroni corrections) are warranted to confirm the stability of these significant findings

Testicular tissue retained normal architecture across all experimental groups: seminiferous tubules and Leydig cells remained structurally intact, and neither basement membrane thickening nor cellular atrophy occurred. These observations contrast with earlier studies attributing oxidative stress, apoptotic cascades, and structural tissue injury to opioid exposure (Elliot et al., 2012; Hamed et al., 2023), a discrepancy potentially driven by variations in dosing regimens and treatment duration. The structural integrity observed here highlights a paradigm of subclinical reproductive toxicity, where physiological disruptions occur prior to detectable tissue pathology. Because histological evaluation was limited to qualitative assessment, future studies should incorporate semi-quantitative

methods such as the Johnsen scoring system alongside morphometric measurements (e.g., tubule diameter and epithelial height) for objective cross-group comparisons. Because the decoupling of structural preservation from sperm and hormone dysregulation is a central insight of this work, integrating quantitative histopathology will be essential to confirm these outcomes. The photomicrographs presented (Plates 1–5) are representative of the histological features that were consistently observed across all five animals examined per group.

The preserved histoarchitecture of the testes as seen in the present study as well as the changes in sperm parameters that were observed including increased sperm count in some groups and reduction in sperm motility is some groups, and the insignificant decrease seen in the body weight and testosterone may be a pointer to latent reproductive response. Considering the literature, the results of this study are consistent with opioid effects on the hypothalamic–pituitary–gonadal axis and testicular function (Duca *et al.*, 2019), and with the recent fentanyl-rat study showing increased sperm count but evidence of testicular stress (Kosal *et al.*, 2025). The upward trend in sperm count could be interpreted as compensatory spermatogenesis in response to gonadal/endocrine disturbance, or may indicate differential sensitivity of sperm output vs sperm quality/motility.

CONCLUSION

This study demonstrates that treatment of adult male Wistar rats with fentanyl at increasing doses for 21 d resulted in slight increase in body weight with an observed reduction in testosterone levels, an increase in sperm count, and mixed effects on motility and morphology, while testicular histology remained preserved. These findings suggest that fentanyl may exert subtle and early reproductive effects which are subclinical in nature and undetected by conventional histological assessment. There is therefore need for use of more sensitive biomarker-based approaches in reproductive toxicology which supports the use of such models in preclinical drug safety analysis and reproductive risk evaluation.

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CONFLICT OF INTEREST

The author declares no conflict of interest.

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