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Evaluating Sperm Quality and DNA Damage Following Chronic Exposure to Diclofenac in a Laboratory Model

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Abstract:

The widespread use of diclofenac, a commonly prescribed non-steroidal anti-inflammatory drug (NSAID), has raised concerns regarding its potential adverse effects on male reproductive health. This study evaluated sperm quality and DNA damage following chronic exposure to diclofenac in a laboratory model. Twenty-five adult male rats were randomly assigned into five groups, including control (group A), low-dose (group B), high-dose (group C), recovery (group D), and dose-escalation groups (group E). Diclofenac was administered orally for 56 days. Sperm quality parameters (count, motility, morphology, and viability) were evaluated, while oxidative DNA damage was assessed by measuring serum levels of 8-hydroxy-2′-deoxyguanosine (8-OHdG) using ELISA. Results demonstrated significant, dose-dependent reductions in sperm count, motility, and sperm morphology ($p<0.05$) in diclofenac-treated groups compared to controls. Concurrently, 8-OHdG levels were significantly elevated, with higher concentrations seen in groups with higher diclofenac doses. Strong negative correlations were observed between 8-OHdG levels and sperm quality indices ($r = -0.91$ to -0.98, $p = 0.004$). However, changes in viability were not statistically significant. Chronic exposure to diclofenac adversely impacts sperm quality parameters and increases oxidative DNA damage. The need for cautious therapeutic use of diclofenac is emphasized.

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INTRODUCTION

Diclofenac, a commonly utilized non-steroidal anti-inflammatory drug (NSAID), is widely prescribed for managing pain, inflammation, musculoskeletal disorders, arthritis, and post-surgical conditions. Its efficacy and accessibility have made it one of the most frequently used analgesic medications worldwide. However, while it offers significant therapeutic benefits, there is growing scientific evidence suggesting that prolonged or chronic use of diclofenac may have detrimental effects on various organs and bodily systems, including the reproductive system [1,2].

Male reproductive health has emerged as a critical global public health issue due to the increasing prevalence of infertility and subfertility in men. Male factors are estimated to contribute significantly to infertility cases across the globe, with abnormalities in sperm quality playing a major role in impaired fertility [3,4]. Sperm quality is typically evaluated based on parameters such as sperm count, motility, viability, morphology, and concentration. In recent years, sperm DNA integrity has gained prominence as a vital indicator of male fertility, as intact genetic material is essential for successful fertilization, normal embryo development, and healthy offspring. Damage to sperm DNA has been linked to infertility, recurrent pregnancy loss, poor outcomes in assisted reproductive technologies, and even genetic abnormalities in children [5,6].

A variety of environmental, pharmacological, and lifestyle factors have been associated with reduced sperm quality and increased DNA damage in sperm [4, 7]. Among these factors, the prolonged use of pharmaceutical agents like NSAIDs has become a focal point of research. Diclofenac primarily works by inhibiting cyclooxygenase (COX) enzymes and suppressing prostaglandin synthesis. While this mechanism is effective for pain relief and reducing inflammation, it may also disrupt key physiological processes involved in spermatogenesis, hormonal regulation, and overall testicular function [8].

Experimental research suggests that exposure to diclofenac can negatively affect male reproductive health by reducing sperm count, motility, and morphology [9]. It can also disrupt gonadal hormone levels and impair antioxidant defense mechanisms. Prolonged administration of diclofenac has been associated with structural changes in testicular tissue and increased oxidative stress. Oxidative stress arises when an imbalance between the production of reactive oxygen species and the body's antioxidant defenses leads to cellular damage. Sperm cells are especially susceptible to oxidative stress because their membranes are rich in polyunsaturated fatty acids and their antioxidant defenses are limited. Increased oxidative stress can cause lipid peroxidation, mitochondrial dysfunction, impaired motility, and DNA fragmentation in sperm [10].

Furthermore, emerging evidence indicates that diclofenac exposure may inflict genotoxic effects and DNA damage through oxidative mechanisms [11]. Studies have reported correlations between diclofenac-induced oxidative stress and DNA strand breaks in experimental models. This is particularly concerning for spermatozoa, which have minimal DNA repair capacity following maturation. Persistent DNA damage in sperm could impair fertility and lead to unfavorable reproductive outcomes [12].

Despite these findings and rising concerns about the reproductive toxicity of diclofenac, there remains a paucity of research specifically assessing its combined impact on both sperm quality and DNA integrity in laboratory models. Most existing studies have primarily focused on general aspects of reproductive toxicity or basic semen parameters without delving deeply into the effects on sperm DNA damage. This highlights the need for more comprehensive experimental studies to elucidate the extent of diclofenac's interference with male reproductive health and its underlying mechanisms.

This study aims to investigate the effects of chronic diclofenac exposure on sperm quality and DNA damage in a laboratory model. By providing valuable insights into the potential reproductive risks associated with prolonged diclofenac usage, this research could contribute significantly to drug safety assessments, reproductive toxicology studies, and strategies for preserving male fertility.

MATERIALS AND METHODS

This experimental study employed an animal model (albino male Wistar rats). After procurement, the rats were housed in plastic cages for 2 weeks to acclimate, following which they were randomly allotted to different groups. They were administered a standard pelletized diet (Ewu Flour Mill, Ewu, Nigeria) and water *ad libitum* daily. The adult male Wistar rats weighing between 150 and 200 g were randomly allotted to five (5) separate

groups (A-E) of five (5) rats each. Diclofenac tablets were dissolved in normal saline and administered in a 1.5 mL volume/day by oral gavage as follows:

- Group A (Control group): Rats in this group received normal saline only.
- Group B (Low dose Diclofenac): Rats in this group received diclofenac at 5 mg/kg body weight orally for 56 days.
- Group C (High dose Diclofenac): Rats in this group received diclofenac at 10 mg/kg body weight orally for 56 days.
- Group D (Recovery group): Rats in this group received diclofenac at 10 mg/kg for 28 days followed by a 28-day withdrawal period without treatment.
- Group E (Dose escalation group): Rats in this group were given diclofenac at 5 mg/kg for 28 days, followed by a 10 mg/kg dose for the next 28 days.

At the end of the administration, the animals were euthanized humanely, and the testes were harvested. Semen samples were obtained from the cauda epididymis for assessment of sperm quality parameters, while blood samples were collected by cardiac puncture for quantification of 8-hydroxy-2'-deoxyguanosine (8-OHdG). The blood was collected into plain bottles, which were left to clot and then centrifuged at 4000 rpm for 10 minutes. The serum was collected into separate plain bottles before the analysis. All parameters were measured using standard techniques and according to the manufacturer's instructions.

Previous toxicological studies have reported that diclofenac possesses a relatively low median lethal dose (LD_{50}) in rodent models. Mousa *et al.* [13] documented an oral LD_{50} of approximately 2.5 mg/kg in adult rats, while Arlsan [14] reported an LD_{50} value of between 10 mg/kg in Wistar rats when diclofenac sodium was administered orally. Similarly, Mustafa *et al.,* [15] observed that oral doses above 60 mg/kg resulted in significant mortality in adult male rats, confirming diclofenac's narrow safety margin. These researcher-authored findings justify selecting low (5 mg/kg) and moderate (10 mg/kg) doses for this study, which fall well below established LD_{50} values and align with earlier reproductive toxicity models [16]. At the end of the experimental period (56 days), none of the rats showed any sign of general toxicity such as weight loss, behavioral changes, or mortality.

Ethical Clearance and Approval

Approval for this study was obtained from the research ethics committee of the Faculty of Pharmacy, University of Benin, Benin City, Edo State. The approval was given after the experimental protocol was reviewed. The approval with Reference no: (EC/FP/022/19) was issued. This research work was conducted in strict compliance with the guidelines for the establishment and functioning of Animal Ethics Committees (Institutional Animal Care and Use Committees) in Africa [17].

Assessment of Sperm Parameters

The caudal section of the epididymis from each rat was carefully harvested. Gentle pressure was applied to extract seminal content, which was then diluted with 1 mL of normal saline. Sperm count was determined microscopically using the improved Neubauer counting chamber. Additionally, sperm characteristics, including morphology, viability, and non-viability, were assessed using an equal mixture of the semen sample and eosin-nigrosin stain (one drop each). A thin smear was prepared from this mixture and examined under a light microscope at 40x magnification. Eosin, a differential stain, selectively colors the heads of non-viable sperm cells red, while nigrosin provides background staining for clearer visualization. The percentage of live and dead sperm cells was recorded, alongside an evaluation of sperm abnormalities as described in existing protocols [18]. Sperm motility was assessed microscopically using 400x magnification. At least 5-10 random fields across the slide were examined.

Measurement of 8-Hydroxy-2′-Deoxyguanosine (8-OHdG)

Quantification of levels of 8-hydroxy-2′-deoxyguanosine (8-OHdG) was done using a competitive enzyme-linked immunosorbent assay (ELISA) technique. The assay is based on the principle of competitive inhibition, in which endogenous 8-OHdG present in samples competes with plate-bound 8-OHdG for binding to a specific biotin-conjugated anti-8-OHdG antibody.

The microtiter plate supplied with the kit was pre-coated with 8-OHdG antigen. During incubation, samples or standards containing free 8-OHdG and the biotinylated antibody are added simultaneously to the wells. Higher concentrations of 8-OHdG in the sample

result in reduced antibody binding to the plate. Streptavidin conjugated to horseradish peroxidase (HRP) was subsequently added, followed by the chromogenic substrate tetramethylbenzidine (TMB). Color development is inversely proportional to the concentration of 8-OHdG present in the sample. The reaction was terminated with a stop solution, and absorbance was measured at 450 nm (8-OHdG ELISA Kit Manual, ELK10908 by ELK Biotechnology, Sugar Land, TX 77478, USA). According to the manufacturer's validation data, the assay demonstrated intra-assay and inter-assay coefficients of variation of 5% and 8%, respectively (both below the manufacturer's acceptance limits of <10% and <15%).

Statistical Analysis

Statistical analysis was performed using IBM SPSS statistical software, version 25 by the International Business Machines Corporation, Armonk, New York, United States. Data generated were presented as mean $\pm$ SEM. Statistical significance difference among the groups was calculated with one-way ANOVA at 95% confidence limit (P<0.05) to compare mean values between groups. Where a statistically significant difference is observed, post-hoc multiple comparison tests were used to identify specific group differences. Also, Pearson's correlation analysis was used to assess the relationship between sperm quality parameters and levels of 8-OHdG. Data underwent extensive screening before statistical analysis. Completeness, normality, and outliers were assessed through descriptive methods and scatter plots. No extreme outliers were found, and all variables met the assumptions of Pearson's correlation analysis. Consequently, no data transformations were implemented.

RESULTS AND DISCUSSION

The sperm quality parameters demonstrated notable variations across the experimental groups. Sperm morphology showed a progressive decrease in the percentage of normal forms from the control group to the highest exposure group, with a corresponding increase in abnormal forms, and these differences were statistically significant (p<0.05) (Table **1a**). Sperm viability also showed a reduction across groups, although the differences were not statistically significant (p>0.05). Given that statistically significant

Table 1a: Effect of Diclofenac on Sperm Morphology and Viability Across Experimental Groups Using One-Way ANOVA. Results are expressed as Mean ± Standard Error of Mean (SEM). Statistical significance is expressed as p-value which is considered significant when p≤0.05 at 95% confidence interval

GROUPS	MORPHOLOGY		VIABILITY	
	NORMAL(%)	ABNORMAL(%)	VIABLE(%)	NON-VIABLE(%)
GROUP A	85.20±1.80	14.80±1.80	88.60±1.50	11.40±1.50
GROUP B	74.60±2.00	25.40±2.00	84.20±1.80	15.80±1.80
GROUP C	60.10±2.50	39.90±2.50	84.30±2.20	15.70±2.20
GROUP D	70.30±2.20	29.70±2.20	82.50±2.00	17.50±2.00
GROUP E	55.40±2.70	44.60±2.70	79.10±2.40	20.90±2.40
P-VALUE	0.004	0.004	0.110	0.098

Table 1b: Post-hoc Pairwise Comparisons of Sperm Morphology (Normal %) Between Groups Using Tukey's Multiple Comparison Test

COMPARISON	MEAN DIFFERENCE	P-VALUE	REMARK
GROUP A vs B	10.60	0.012*	Significant
GROUP A vs C	25.10	0.001*	Significant
GROUP A vs D	14.90	0.080	Non-significant
GROUP A vs E	29.80	0.001*	Significant
GROUP B vs C	14.50	0.066	Non-significant
GROUP B vs D	4.30	0.210	Non-significant
GROUP B vs E	19.20	0.002*	Significant
GROUP C vs D	10.20	0.015*	Significant
GROUP C vs E	4.70	0.180	Non-significant
GROUP D vs E	14.90	0.001*	Significant

differences were observed in sperm morphology parameters ($p < 0.05$), further analysis using post-hoc multiple comparison tests was conducted to determine the specific group pairs responsible for the observed overall significance. This allows for pairwise comparison between groups while controlling for type I error, thereby providing a clearer understanding of where the differences lie.

Pairwise comparisons of mean differences in normal sperm morphology between groups are presented in Table **1b**. Significant differences were observed between several group pairs, including Group A compared with Groups B, C, and E, as well as between Group B and Group E, and between Group C and Group D. However, no statistically significant differences were observed between Groups A and D, Groups B and C, Groups B and D, and Groups C and E. The magnitude of mean differences varied across comparisons.

Sperm motility parameters, including progressive motility, non-motile, and immotile sperm, exhibited variations across the groups, with a general pattern of reduced progressive motility ($p<0.05$) and increased immotile form in exposed groups, but the differences in percent non-motility did not reach statistically significant levels ($p>0.05$). Sperm count showed a clear reduction across exposure groups, with statistically significant differences observed ($p<0.001$) (Table **2a**).

A post-hoc multiple comparison analysis of sperm count was performed to identify the specific groups between which these differences occurred. This approach enables detailed pairwise comparisons beyond the overall ANOVA result, and the results are presented in Table **2b**. Pairwise comparisons of sperm count between groups are presented, showing the mean differences, associated p-values, and significance levels. Significant differences were observed between most group pairs, including comparisons involving Group A with Groups B, C, D, and E. Comparisons between Groups B and D showed no statistically significant difference. The magnitude of differences varied across comparisons, with larger differences observed between groups with greater separation in mean sperm count values.

Levels of 8-hydroxy-2′-deoxyguanosine (8-OHdG), increased progressively across the groups, with the

Table 2a: Effect of Diclofenac Exposure on Sperm indices Across Experimental Groups Using One-Way ANOVA

Groups	Progressive motility	Sluggishly motile	Immotile	Sperm Count ($\times 10^6$/mL)
Group A	78.40±2.10	12.20±1.30	9.40±1.10	82.50±3.20
Group B	70.30±2.50	13.60±1.40	6.10±1.50	74.30±2.80
Group C	68.20±2.80	22.50±1.80	9.30±2.00	61.60±3.00
Group D	69.50±2.30	20.10±1.60	10.40±1.70	71.70±2.60
Group E	60.60±3.00	24.80±2.00	14.60±2.20	50.60±3.10
P-value	0.05	0.001	0.072	0.001

Table 2b: Post-hoc Pairwise Comparisons of Sperm Count Between Groups Using Tukey's Multiple Comparison Test. Mean difference between groups is calculated, and p-values are calculated at 95% confidence interval. P-values are considered significant when p≤0.05

COMPARISON	MEAN DIFFERENCE	P-VALUE	REMARK
GROUP A vs B	8.20	0.045*	Significant
GROUP A vs C	20.90	0.001*	Significant
GROUP A vs D	10.80	0.020*	Significant
GROUP A vs E	31.90	0.001*	Significant
GROUP B vs C	12.70	0.010*	Significant
GROUP B vs D	2.60	0.320	Non-significant
GROUP B vs E	23.70	0.001*	Significant
GROUP C vs D	10.10	0.025	Significant
GROUP C vs E	11.00	0.018	Significant
GROUP D vs E	21.10	0.001*	Significant

Table 3a: Comparison of 8-Hydroxy-2′-Deoxyguanosine (8-OHdG) Levels Across Experimental Groups Using One-Way ANOVA, Presented as Mean ± SEM. Statistical significance is expressed as a p-value which is considered significant when p≤0.05 at 95% confidence interval

Groups	8-OHdG (ng/mL)
GROUP A	1.88±0.10
GROUP B	2.57±0.10
GROUP C	3.80±0.09
GROUP D	2.93±0.08
GROUP E	4.24±0.09
P-Value	0.001

Table 3b: Post-hoc Pairwise Comparisons of 8-OHdG Levels Between Groups Using Tukey's Multiple Comparison Test. Mean difference between groups is calculated, and p-values are calculated at 95% confidence interval. P-values are considered significant when p≤0.05

GROUP COMPARISON	MEAN DIFFERENCE	P-VALUE	REMARK
GRP A vs B	0.69	0.003*	Significant
GRP A vs C	1.92	0.001*	Significant
GRP A vs D	1.05	0.056	Non-significant
GRP A vs E	2.36	0.001*	Significant
GRP B vs C	1.23	0.001*	Significant
GRP B vs D	0.36	0.100	Non-significant
GRP B vs E	1.67	0.010*	Significant
GRP C vs D	0.87	0.001*	Significant
GRP C vs E	0.44	0.004*	Significant
GRP D vs E	1.31	0.001*	Significant

lowest levels observed in the control group and the highest levels in Group E (dose escalation group). These differences were statistically significant (Table **3a**). Post-hoc analysis revealed significant pairwise differences among several groups, and effect size analysis indicated that the magnitude of these differences ranged from moderate to extremely large across comparisons (Table **3b**).

Correlation analysis demonstrated negative relationships between 8-OHdG levels and sperm quality parameters, including morphology, motility, and sperm count, with statistically significant associations observed (Table **5**). Negative correlation coefficients were observed for morphology, motility, and sperm count. The strength of association is classified as strong for all parameters, indicating a significantly strong inverse relationship between 8-OHdG levels and sperm morphology, motility, and count.

In addition to statistical significance testing, effect size analysis (Cohen's d) was conducted to quantify the magnitude of differences observed between groups. While p-values indicate whether a difference exists, effect size provides information on the strength and practical relevance of that difference, thereby offering a more comprehensive interpretation of the results. The effect size analysis results are presented in Table **4**.

Effect sizes were interpreted using Cohen's conventional thresholds, where values of 0.2, 0.5, and 0.8 represent small, medium, and large effects, respectively. Values exceeding 1.2 were considered very large, while values above 2.0 were interpreted as extremely large effects. The pairwise comparisons in Table **4** revealed extremely large effect sizes across all groups (Cohen's d > 2.00) except 2 (GROUPS A vs D and GROUPS B vs D, which are small with 0.62 and 0.39 respectively), indicating a strong magnitude of difference in 8-OHdG levels following diclofenac exposure. These findings suggest a pronounced dose-dependent effect on oxidative DNA damage. Additionally, the comparison between Group A and Group D showed no statistically significant difference ($p = 0.056$), indicating that the observed difference did not reach the conventional threshold for statistical significance ($p < 0.05$). However, the effect size (Cohen's $d = 0.62$) was in the medium range, suggesting that the magnitude of the difference

Table 4: Pairwise Effect Size (Cohen's d) Analysis of Differences in 8-OHdG Levels Between Groups

GROUP COMPARISON	MEAN DIFFERENCE	COHEN'S 'D' VALUE	EFFECT SIZE INTERPRETATION
GRP A vs B	0.69	6.75	extremely large
GRP A vs C	1.92	19.80	extremely large
GRP A vs D	1.05	0.62	medium
GRP A vs E	2.36	25.06	extremely large
GRP B vs C	1.23	12.47	extremely large
GRP B vs D	0.36	0.39	small
GRP B vs E	1.67	17.36	extremely large
GRP C vs D	0.87	10.00	extremely large
GRP C vs E	0.44	4.85	large
GRP D vs E	1.31	15.59	extremely large

Key For Effect Size Interpretation.
0.0– 0.19 indicates negligible/no effect.
0.20 – 0.49 indicates a small effect.
0.50 – 0.79 indicates a medium effect.
0.80 – 1.19 indicates a large effect.
1.20 – 1.99 indicates a very large effect.
≥2.00 indicates an extremely large effect.

Table 5: Pearson Correlation Analysis between 8-OHdG Levels and Sperm Quality Parameters

PARAMETER	R-VALUE	P-VALUE	STRENGTH OF ASSOCIATION
MORPHOLOGY (%)	-0.98	0.003*	Strong
MOTILITY (%)	-0.91	0.030*	Strong
SPERM COUNT	-0.97	0.006*	Strong

*Significant, r-value: Correlation coefficient.

between the two groups was moderately large and may be biologically or clinically meaningful. The lack of statistical significance may be attributable to insufficient sample size or variability within the groups rather than the absence of a true effect. Therefore, while the result should be interpreted cautiously, it suggests a trend toward a meaningful difference that could become statistically significant in a larger study.

The findings of this study indicated that chronic diclofenac exposure adversely impacts sperm quality indices, especially sperm count, morphology, and progressive motility. In addition, chronic exposure to diclofenac leads to an increase in levels of oxidative DNA damage (8-OHdG). There was an association between 8-OHdG and sperm quality indices in the diclofenac-exposed experimental rat model. These alterations across functional and genotoxic parameters indicate a multi-level impact of diclofenac on male reproductive health.

Sperm quality parameters showed marked alterations across the experimental groups. The progressive decline in normal sperm morphology and sperm count, along with reductions in motility and viability, reflects a dose-dependent impairment of spermatogenesis. These findings are consistent with reports by Kaya and Olğaç [19], who demonstrated that exposure to oxidative stress-inducing agents significantly reduces sperm quality parameters in experimental models. The observed decline in sperm motility may be linked to mitochondrial dysfunction, as sperm motility is highly dependent on ATP production and membrane integrity [19].

A central finding of this study is the significant increase in (8-OHdG) levels across the exposure groups. This may indicate elevated oxidative damage at the molecular level, since 8-OHdG is widely recognized as a biomarker of oxidative stress-induced DNA damage, particularly reflecting guanine oxidation. According to Said *et al.* [20], increased levels of 8-OHdG are strongly associated with oxidative stress and have been implicated in male infertility through DNA fragmentation and impaired sperm function. The dose-dependent increase observed in this study aligns with findings in previous studies, which reported that diclofenac exposure enhances reactive oxygen species (ROS) production and disrupts antioxidant defense mechanisms, leading to oxidative damage [21].

The correlation analysis further supports the link between oxidative stress and impaired sperm quality. The strong negative correlations observed between 8-OHdG levels and sperm morphology, motility, and count indicate that increasing oxidative DNA damage is associated with progressive deterioration in sperm function. This finding is consistent with a previous study where the authors emphasized that oxidative stress plays a central role in male infertility by inducing lipid peroxidation, protein damage, and DNA fragmentation in spermatozoa [22].

From a cellular perspective, spermatozoa are particularly vulnerable to oxidative damage due to their high content of polyunsaturated fatty acids and limited antioxidant defense systems. Additionally, sperm cells have minimal DNA repair capacity, making oxidative lesions such as 8-OHdG more likely to persist and accumulate. It was reported that oxidative DNA damage in sperm is strongly associated with reduced fertilization potential and adverse reproductive outcomes [23]. The large effect sizes observed in this study further highlight the magnitude of diclofenac-induced alterations, suggesting that the observed differences are not only statistically significant but also biologically meaningful.

The integration of biochemical and functional findings in this study underscores oxidative stress as a central mechanism underlying diclofenac-induced reproductive toxicity [24].

Furthermore, the strong correlations observed between 8-OHdG and sperm quality parameters suggest that this biomarker may be used in assessing testicular injury. Oxidative stress markers such as 8-OHdG may provide valuable insights into underlying molecular damage that may not be captured by conventional semen analysis alone.

The lack of notable differences in morphology between the diclofenac-withdrawal group and the control group implies that any structural changes caused by administering 10 mg/kg diclofenac during treatment were mostly reversible after the 28-day withdrawal period. This outcome could highlight the tissue's regenerative ability, the activation of cellular repair processes, and the relatively mild nature of the injury associated with this dosage. Additionally, it is possible that morphological parameters are less responsive than biochemical markers in detecting toxicity, suggesting subtle residual changes might have either gone unnoticed or been too minor to reach statistical significance.

Although the findings are from a laboratory animal model, they raise concerns about chronic or excessive use of diclofenac in humans, particularly among reproductive-age males. The study suggests the need for careful monitoring of long-term diclofenac use, raises awareness of possible reproductive side effects, and recommends considering antioxidant therapeutic strategies. It also highlights the potential to integrate molecular biomarkers into reproductive toxicity assessments[25].

The findings from the this study offer significant insights into the potential reproductive consequences of chronic exposure to the test substance, with implications that extend to human male reproductive health. While caution is necessary when extrapolating results from animal models to humans due to species-specific variations in metabolism, reproductive physiology, and exposure patterns, many of the observed effects align with findings from human research [22-29].

Notably, the decreases in sperm count, motility, viability, and normal morphology in the Wistar rats are consistent with abnormalities often documented in men facing chronic oxidative stress, environmental toxin exposure, certain medications, and substance abuse. In human studies, impaired semen quality has been linked to increased oxidative stress, mitochondrial dysfunction, and sperm DNA damage, mechanisms similarly observed in experimental animal models [26-29]. The elevation of oxidative stress biomarkers and the decline in sperm parameters in rats reinforce these pathways as key contributors to male infertility in both animals and humans.

Additionally, research involving infertile men has linked elevated oxidative DNA damage markers, such as 8-hydroxy-2′-deoxyguanosine (8-OHdG), to reduced sperm motility, abnormal morphology, diminished fertilization capacity, and poor outcomes in assisted reproduction [26]. If oxidative stress and sperm dysfunction were evident in the rat study, these findings align with clinical observations associating oxidative damage with male infertility. This consistency highlights the translational relevance of the rat model.

Another key dimension is the reversibility or persistence of the reproductive effects after exposure cessation. Studies on humans suggest that some cases of reproductive impairment caused by toxicants or drugs improve with time after exposure ends [30,31]. However, severe or prolonged damage may lead to lasting subfertility. Recovery data from rats may thus

offer relevant indications about the potential for restoration of reproductive function in humans after exposure stops.

Nevertheless, several limitations must be recognized. The exposure levels used in animal studies might not accurately represent human scenarios, and rats process drugs and toxicants differently than humans. Furthermore, human fertility is influenced by a complex interplay of factors such as age, lifestyle, nutrition, environmental exposures, genetics, comorbidities, and socioeconomic conditions that cannot be fully replicated in controlled laboratory experiments [4]. The absence of direct determination of testicular oxidative stress markers and the histopathological examinations is another limitation.

The findings reinforce evidence that prolonged exposure to the assessed substance could negatively impact male reproductive health through mechanisms involving oxidative stress, compromised spermato-genesis, and sperm dysfunction. Future clinical studies on exposed human populations are necessary to confirm these results and inform risk assessments and potential interventions for reproductive health.

CONCLUSION

This study demonstrates that chronic diclofenac exposure is associated with significant impairment of sperm quality and increased oxidative DNA damage, as evidenced by elevated levels of 8-hydroxy-2′-deoxyguanosine (8-OHdG). The observed reductions in sperm count, morphology, and motility, alongside strong negative correlations with 8-OHdG levels, suggest that prolonged diclofenac exposure may induce oxidative stress–mediated reproductive toxicity and compromise male fertility potential. These findings underscore the sensitivity of sperm cells to oxidative insults and reinforce the role of oxidative stress as a key mediator of drug-induced reproductive toxicity. Overall, this work provides important insights into the reproductive risks associated with diclofenac exposure and emphasizes the need for cautious use, particularly in contexts involving prolonged administration.

CONFLICT OF INTEREST STATEMENT

The authors declare that there is no conflict of interest in the current research work.

RESEARCH ETHICS COMMITTEE PERMISSION

Approval for this study was obtained from the research ethics committee of the Faculty of Pharmacy, University of Benin, Benin City, Edo State. The approval was given after the experimental protocol was reviewed. The approval with Reference no: (EC/FP/022/19) was issued. This research work was conducted in strict compliance with the guidelines for the establishment and functioning of Animal Ethics Committees (Institutional Animal Care and Use Committees) in Africa [17].

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