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Industrial Dioxin as an Endocrine Disruptor: Combined Effects with Hyperglycemia on Male Reproductive Health

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Abstract

Background: Industrial pollutants like dioxins, known endocrine disruptors, pose significant risks to reproductive health, particularly when combined with metabolic disorders such as hyperglycemia. This study evaluated the synergistic effects of dioxin exposure and hyperglycemia on testicular function in male Wistar rats. **Objectives:** To assess the independent and synergistic effects of hyperglycemia and dioxin (TCDD) exposure on testicular function, spermatogenesis, and oxidative balance. **Methodology:** Twenty male Wistar rats were divided into four groups: normal control, diabetes only (STZ-induced), dioxin only, and diabetes combined with dioxin. Fasting blood glucose, body weight, serum testosterone, sperm parameters, oxidative stress markers (MDA and SOD), and testicular histology were assessed. Histological sections were analyzed using hematoxylin-eosin and Masson's trichrome staining. **Results:** Diabetic and diabetic+dioxin groups exhibited elevated blood glucose, though differences were not statistically significant. Body weight increased significantly in the diabetic group but decreased markedly with dioxin exposure. Testosterone levels rose in diabetes alone but were significantly suppressed by dioxin, especially in the combined exposure group. Sperm motility, count, and viability were significantly reduced in diabetic and diabetic+dioxin groups, while dioxin alone paradoxically increased motility and viability. MDA levels were lowest, and SOD activity was severely reduced in dioxin-exposed groups, indicating heightened oxidative stress. Histological analyses revealed pronounced degeneration, germinal epithelium thinning, and extensive interstitial fibrosis in the diabetic+dioxin group, confirming synergistic testicular toxicity. **Conclusion/ Recommendations:** Combined exposure to hyperglycemia and dioxin exerts a synergistic, deleterious effect on male reproductive health by exacerbating oxidative stress, impairing spermatogenesis, and promoting fibrotic remodelling. These findings underscore the heightened reproductive risks posed by concurrent metabolic and environmental insults.

Keywords: Streptozotocin**,** Diabetes mellitus**,** Oxidative stress**,** Malondialdehyde**,** Masson's trichrome**.**

Introduction

Male infertility remains a significant global health concern, with male factors implicated in up to 50% of infertility cases among couples (Abbasi *et al.,* 2020). Infertility is a significant public health concern, affecting reproductive rights, mental health, and social well-being, while also burdening healthcare systems and economies through increased demand for medical interventions and reduced population growth potential (WHO, 2023). Oxidative stress, endocrine disruption, and environmental toxins are recognized as major contributors to impaired spermatogenesis and

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testicular dysfunction (Wang *et al.,* 2022; Motolla *et al.,* 2024).

Hyperglycemia is the specialized term for high blood glucose, which can be due to a low level of insulin or resistance of the body to insulin. Production of lactate from glucose by Sertoli cells is necessary for the maintenance of spermatogenesis. Therefore, blood-to-germ cell transport of glucose and other metabolic intermediates from the basal to the adluminal compartment is highly controlled, particularly owing to the presence of the blood-testis-barrier (BTB) (Johnson *et al.,* 2020). Hyperglycemia has been reported to induce testicular damage, therefore impairing testicular function and spermatogenesis (Song *et al.,* 2022). In the testis, hyperglycemia seems to cause increased oxidative stress with increased reactive oxygen species production in seminal fluid (Papachristoforou *et al.,* 2020) and lipoperoxidation (LP), sperm DNA fragmentation (Omolaoye *et al.,* 2022), sperm mitochondrial bioenergy alteration (Song *et al.,* 2022), and enzymatic glycation end products (Darmishonnejad *et al.,* 2024). Molecular investigation techniques have also confirmed that in diabetic men there is a higher percentage of sperm with nuclear and mitochondrial DNA fragmentation, which is due to increased oxidative stress induced by hyperglycemia (Pourheydar *et al.,* 2021; Facondo *et al.,* 2022).

Furthermore, experimental and epidemiological studies have demonstrated that dioxins can exacerbate metabolic disorders by reducing insulin secretion, impairing glucose uptake, and inducing insulin resistance, thereby promoting hyperglycemia (Novelli *et al.,* 2021; Kishi *et al.,* 2025). Despite the well-established individual impacts of hyperglycemia and dioxins on testicular health, there is a paucity of research investigating their combined, potentially synergistic effects on the male reproductive system. Therefore, this study aimed to evaluate the synergistic effects of hyperglycemia and dioxin exposure on testicular histopathology, hormonal profiles, oxidative stress markers, and sperm parameters in male Wistar rats. By elucidating these combined effects, our research provides valuable insights into the reproductive risks posed by simultaneous metabolic and environmental challenges. This study investigates whether dioxin exposure aggravates endocrine and metabolic disturbances in diabetes, with the hypothesis that combined effects impair glucose regulation, testosterone levels, sperm quality, and body weight through disruption of hormonal pathways.

Materials and Methods

Sourcing and preparation of compounds

Streptozotocin and dioxin were procured from Sigma Aldrich (USA). 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) was first dissolved in a small volume of absolute ethanol to prepare a stock solution. The required dose was then diluted with corn oil to achieve the appropriate concentration for gavage. Streptozotocin (STZ) was freshly prepared on the day of administration by dissolving the required amount of powder in cold 0.1 M sodium citrate buffer (pH 4.5). Distilled water, formaldehyde, immunochemistry reagents, normal saline, formo-saline, citrate buffer, histological stains, and blood glucose measurement kits (Iwanegbe *et al.,* 2019).

Experimental animals and care

Twenty (20) Wistar rats weighing 100-120g were obtained for this study. The animals were housed in healthy conditions at a constant temperature with a 12-hour light and dark cycle and nutritionally balanced food and water. They were given normal feed and allowed to drink water freely. They were handled with care. Animals were acclimatised for one week to their new environment before the commencement of experimental work (Augier et al, 2014).

Experimental design

Twenty adult male and female Wistar rats were randomly assigned to four experimental groups, with five animals per group, to investigate the effects of dioxin and streptozotocin (STZ). Group A (normal control) received normal saline. Group B (Diabetes Only) was induced with hyperglycemia

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by a single intraperitoneal injection of streptozotocin at a dosage of 60 mg/kg body weight. Group C (Dioxin with Diabetes) consisted of diabetic animals (induced with 60 mg/kg STZ) that were subsequently administered dioxin at five µg/kg to assess the combined effect of hyperglycemia and dioxin toxicity. Group D (Dioxin Only) was exposed to dioxin at a dosage of 5 µg/kg body weight, administered to evaluate the toxicological impact of dioxin exposure in the absence of hyperglycemia. All experimental protocols and treatment procedures were conducted in accordance with the guidelines set by the Institutional Animal Care and Use Committee (IACUC). The Faculty of Basic Medical Sciences Ethics Review Committee, Osun State University, Osogbo, Nigeria, approved them.

Animal sacrifice and sample collection

Twenty-four hours after the last administration, the experimental rats were sacrificed by cervical dislocation. Blood for biochemical assays was collected from the apex of the left ventricle and dispensed into the red-topped 10 ml bottles. The rat ovaries were harvested and fixed in Bouin's fluid (Rocha-Pereira *et al.,* 2019).

Procedures for induction of hyperglycemia

Streptozotocin (STZ) was dissolved in a citrate buffer at a concentration of around 60 mg/kg. Rats were fasted overnight before the procedure. It was administered intraperitoneally, and animals were monitored closely after injection for signs of distress or adverse reactions. The effect of the streptozotocin on body weight and fasting blood glucose was confirmed every week (Fajarwati *et al.,* 2023).

Measurements of body weights

The weights of the animals were obtained upon arrival, and every week using digital weighing balance scale in order to account for possible results in physical changes in rats upon administration (STZ and Dioxin) every week. The weights are checked for the comparison of possible changes from the initial weight and kept on record (Fajarwati *et al.,* 2023).

Measurements of fasting blood sugar

The blood sugars of overnight-fasted rats (for about 10-12 hours) were measured by using a GLUCOMETER (Accu-check). Blood was obtained by tail vein puncture. The glucose level was monitored weekly and kept on record (Tariq et al., 2021).

Animal sacrifice

After adequate administration and examination of animals, the animals were prepared for cervical dislocation, which was the way the animals were sacrificed to make them insensitive to pain. The animals were pinned on both hands and legs to the dissecting table using surgery pins. The animals were dissected in the middle, and blood was withdrawn from the heart using sterilized syringes and needles; the blood was withdrawn slowly. The blood samples were centrifuged to collect blood plasma. The testes were collected and weighed on the sensitive weighing balance and kept in a bottle filled with formosaline.

Sperm analysis

As soon as the rats were sacrificed, the motility of the epididymis sperm was measured. Using standard techniques as outlined by Osuntokun (2017), sperm progressive motility was assessed. The % motility was measured by counting the quantity of motile spermatozoa per unit area. A counting chamber was used to count the sperm, and the results were reported as millions of sperm per millilitre of suspension. The investigation of sperm viability was conducted using the Akinsomisoye *et al*. (2017) methodology. Eosin/nigrosin stain was used to create a short, homogeneous spermatozoa smear on slides. To determine the percentage of a life/death ratio, one hundred sperm cells were counted on each slide.

Analysis of testosterone hormones

The blood samples were collected into the red-topped sample bottles, centrifuged, and stored in the

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refrigerator at -200°C. The enzyme-linked immunosorbent assay (ELISA) kits that were used were obtained from Sigma Aldrich (USA) and were built on the competitive inhibition enzyme immunoassay methodology. The kit's microtiter plate already has a specific protein pre-coated on it. An anti-testosterone antibody biotin-conjugated was added to the appropriate microplate wells once the addition of standards or samples was made. The TMB substrate solution was then added to each microplate well, followed by the addition of an avidin-horseradish peroxidase (HRP) conjugate, which was then incubated for 45 minutes. The enzyme substrate reaction was stopped using the kits' stop solution, and the colour shift was detected using an ELISA reader that operates at a wavelength of 450 ± 10 nm *(*Alhaji *et al.,* 2023).

Biochemical parameters

In diabetes and dioxin exposure, MDA and SOD markers assess oxidative imbalance, revealing membrane injury, impaired testicular function, and the synergistic effects of hyperglycemia and toxin-induced ROS production. Serum MDA concentration was measured according to the method of Pires *et al.* (2024), with minor modifications as previously reported by Wang (2025), and serum SOD activity was measured according to the methods of Kono (2022).

Histological examination

The testes were fixed in Bouin's solution; the volume of fixative was at least 10× the volume of the sample. For embedding, fixed testicular samples were dehydrated in increasing concentrations of ethanol (70%, 80%, 95%, 100% × 3), cleared in xylene (× 3), and immediately dipped in molten paraffin wax, and finally embedded in molten paraffin wax to make a paraffin block. The tissues were serially sectioned at 5 µm thickness from the paraffin block using the rotary microtome. The sections were transferred to a glass slide. Histological demonstration was carried out in paraffin wax-embedded sections, which were stained with hematoxylin and eosin and Masson's trichrome.

Statistical analysis

Data were analysed using GraphPad Prism version (GraphPad Software, San Diego, CA, USA). Results were expressed as mean ± standard error of mean (SEM). Group differences were assessed by one-way analysis of variance (ANOVA) followed by Dunnett's post hoc test for multiple comparisons against the control group. A p-value < 0.05 was considered statistically significant.

Results

Fasting blood glucose

The result shows the normal control group (Group A) maintained relatively stable glucose levels throughout the study, ranging between 50 and 60 mg/dL.. In contrast, the diabetic-only group (Group B) exhibited a progressive and significant rise, reaching approximately 156 mg/dl by week 5. The dioxin-plus-diabetes group (Group C) also showed elevated glucose levels, peaking at around 100 mg/dl by week 5. In contrast, the dioxin-only group (Group D) demonstrated moderate increases compared to controls, with values stabilising near 70–90 mg/dl. Two-way ANOVA revealed that interaction effects (30.46%), column factor (treatment groups, 22.25%), and row factor (time, 23.93%) were all statistically significant ($p < 0.0001$). Bonferroni posttests indicated no significant difference between the normal control and diabetic groups in weeks 1–3; however, significant differences emerged in weeks 4 ($p < 0.001$) and 5 ($p < 0.001$). Similarly, Group C (dioxin + diabetes) showed significant elevations over controls in weeks 4 ($p < 0.001$) and 5 ($p < 0.001$), while Group D (dioxin only) exhibited a milder but significant increase at week 4 ($p < 0.05$).

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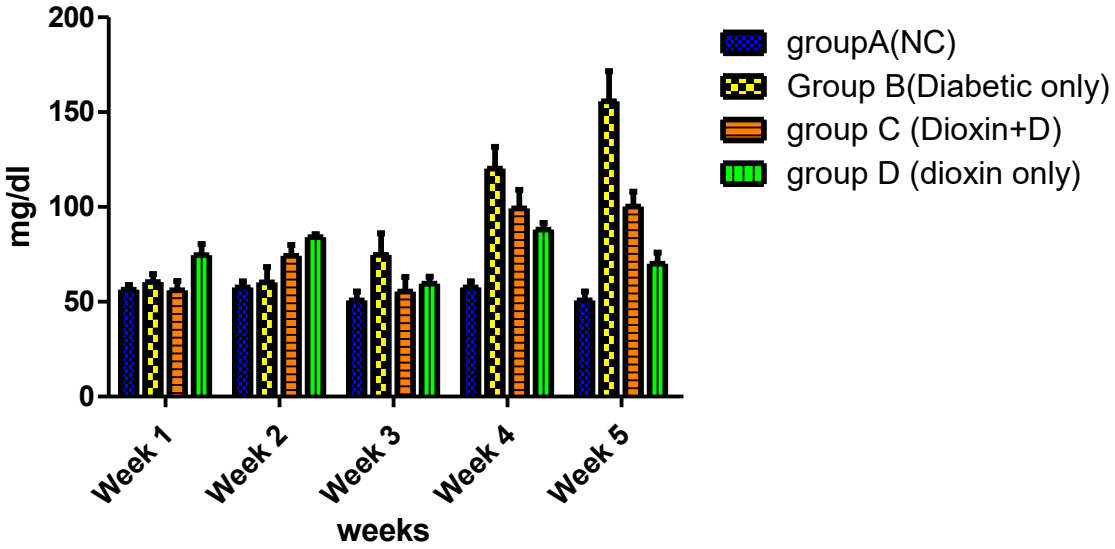

Figure 1: Effect of diabetes and dioxin on fasting blood sugar (FBS) levels over five weeks. The bar graph shows group A maintained stable glucose levels, while group B (diabetic only) showed a significant progressive rise from week 4 onward (***$p < 0.001$). Group C (dioxin + diabetes) also showed significant increases at weeks 4 and 5 (***$p < 0.001$). Group D (dioxin only) demonstrated a moderate rise with significance at week 4 (*$p < 0.05$). Data were analyzed by two-way ANOVA with Bonferroni posttests.

Body weight

Throughout the five weeks, Group B (diabetic only) consistently exhibited the highest body weight, suggesting that diabetes alone promotes weight gain. In contrast, Group A (normal control) showed a moderate increase in weight over time. Groups C and D, which received dioxin either with or without diabetes, displayed lower body weights compared to the control group, indicating a possible suppressive effect of dioxin on weight gain. The lowest body weight values were observed in Group D (dioxin only). Statistical analysis using one-way ANOVA showed a highly significant difference in body weight among the groups ($p < 0.0001$), with an F-value of 32.75 and an R^2 value of 0.8600. This means that 86% of the variation in body weight was due to differences between treatment groups. Bartlett's test for equal variances confirmed that the assumption of homogeneity of variances was met ($p = 0.7734$). Post hoc comparisons using Dunnett's test further clarified these findings. The diabetic-only group (Group B) had a significantly higher body weight than the control group (mean difference = -109.7 g, $p < 0.001$). The group exposed to both diabetes and dioxin (Group C) also showed a significantly lower body weight than the control group (mean difference = -40.16 g, $p < 0.01$). However, the difference between the dioxin-only group (Group D) and the control group did not reach statistical significance (mean difference = -28.40 g, $p > 0.05$).

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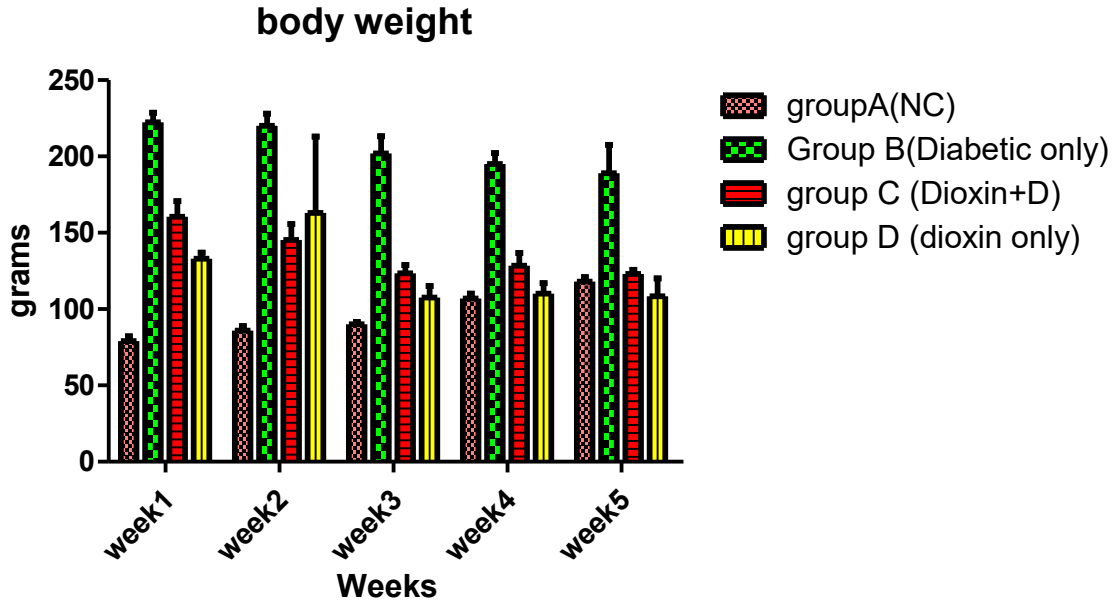

Figure 2: Effect of diabetes and dioxin on body weight over five weeks. The bar graph shows the mean body weight (grams) for each group: Normal Control (Group A), Diabetic Only (Group B), Diabetic + Dioxin (Group C), and Dioxin Only (Group D). Significant differences were determined using one-way ANOVA and Dunnett's post hoc test. **$p < 0.01$, ***$p < 0.001$ vs. Control.

Serum testosterone

In the diabetic-only group, testosterone levels were markedly elevated compared to the normal control, suggesting that diabetes on its own may enhance testosterone production or alter its regulation. On the other hand, subjects exposed to dioxin alone exhibited the lowest testosterone levels among all groups, indicating a potent suppressive effect of dioxin on testosterone. Interestingly, when diabetes and dioxin were combined, testosterone levels were moderately elevated—higher than those in the dioxin-only group but still significantly lower than the diabetic-only group. This suggests that dioxin may partially counteract the testosterone-increasing effect of diabetes. Statistical analysis using one-way ANOVA confirmed that these differences were highly significant ($p < 0.0001$). The F-value was 217.7, with an R-squared value of 0.9761, indicating that nearly all the variation in testosterone levels could be explained by the group differences. However, Bartlett's test for equal variances revealed a significant difference in variances among the groups ($p = 0.0002$), suggesting some variability in the consistency of the responses, particularly in the diabetic-only group. Further comparisons using Dunnett's post hoc test showed that the diabetic-only group had significantly higher testosterone levels than the control group. In comparison, both the dioxin-only and diabetic + dioxin groups had significantly lower levels than the control. These results highlight the contrasting effects of diabetes and dioxin and point to a complex interaction when both factors are present.

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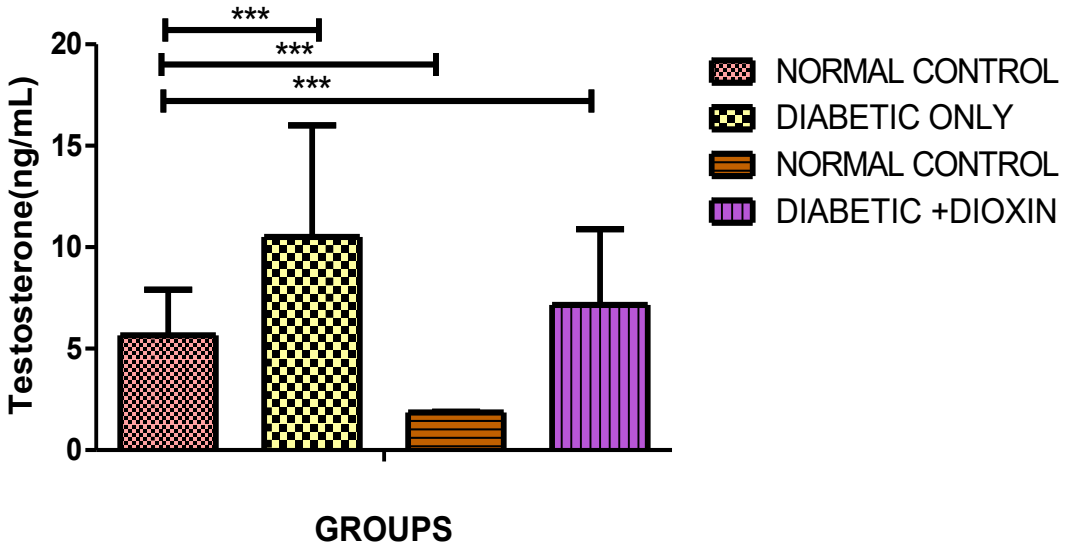

Figure 3: Effect of diabetes and dioxin on serum testosterone levels. The bar graph shows mean testosterone concentration (ng/mL) across four groups: normal control, diabetic only, dioxin only, and diabetic + dioxin. Data were analyzed using one-way ANOVA and Dunnett's test. ***p < 0.001 vs. Normal Control.

Sperm motility

Four experimental groups were evaluated: a normal control group, a group with diabetes only, a group exposed to dioxin only, and a group with both diabetes and dioxin exposure. Sperm motility, represented as a percentage, varied significantly across the groups. The normal control group exhibited relatively high motility. In contrast, the diabetes-only group showed a clear reduction in sperm motility, indicating that diabetes alone has a detrimental effect. Interestingly, the group exposed to dioxin alone displayed an increase in sperm motility compared to the control group. However, when diabetes and dioxin were combined, sperm motility dropped even further than in the diabetes-only group, suggesting a compounding negative effect. Statistical analysis using one-way analysis of variance (ANOVA) confirmed that the differences among the groups were highly significant, with a p-value less than 0.0001. The F value was 130.6, and the R-squared value was 0.9608, indicating that approximately 96% of the variation in sperm motility could be attributed to the group differences. Bartlett's test for equal variances yielded a p-value of 0.4492, confirming that the assumption of equal variances was met and validating the use of ANOVA. Further analysis using Dunnett's multiple comparison test revealed that all experimental groups differed significantly from the control group. The diabetes-only group showed a significant decrease in sperm motility, with a mean difference of -4.980 and a p-value less than 0.001. The dioxin-only group showed a significant increase in motility, with a mean difference of 6.430 ($p < 0.001$). The combination of diabetes and dioxin resulted in the most substantial decrease in motility, with a mean difference of -6.260 ($p < 0.001$).

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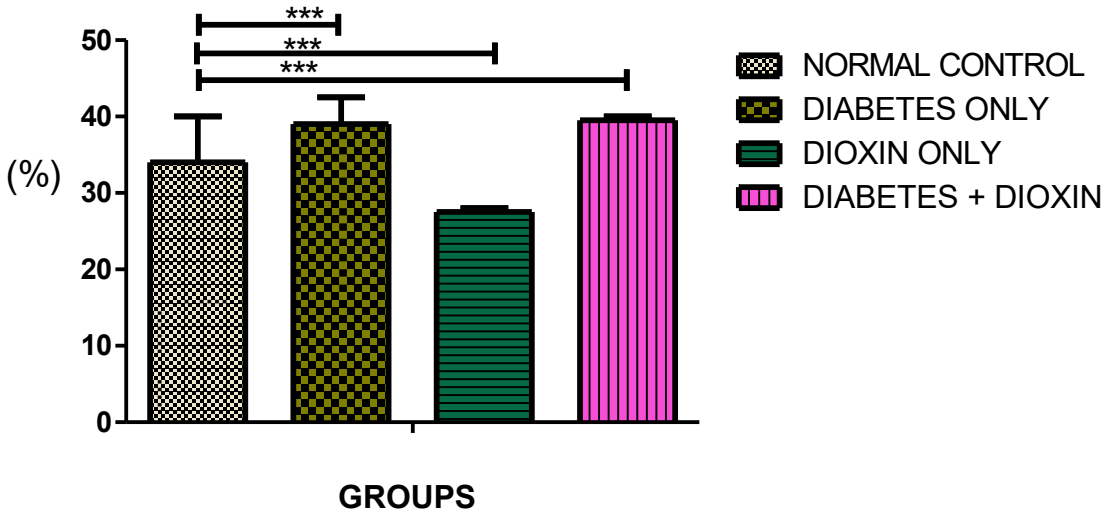

Figure 4: Effect of diabetes and dioxin on sperm motility. The bar graph shows mean sperm motility across four groups: normal control, diabetes only, dioxin only, and diabetes + dioxin. Data were analysed using one-way ANOVA followed by Dunnett's test. ***p < 0.001 vs. Normal Control.

Sperm count

Sperm count was assessed in four experimental groups: normal control, diabetes only, dioxin only, and diabetes + dioxin. The normal control group exhibited a baseline sperm count within the normal physiological range. In contrast, the diabetes-only group showed a marked reduction in sperm count compared to the control. A similar decrease was also observed in the Diabetes + Dioxin group. However, the dioxin-only group showed a slight, non-significant reduction in sperm count relative to the control group. Statistical analysis using one-way ANOVA confirmed that the observed differences in sperm count among the groups were statistically significant, with a p-value of 0.0113 and an F-statistic of 5.118. The R-squared value of 0.4897 indicated that approximately 49% of the variability in sperm count could be attributed to the treatment conditions. Bartlett's test showed no significant difference in variances across the groups, supporting the reliability of the ANOVA results. Post hoc comparison using Dunnett's test revealed that the sperm count in the Diabetes Only group was significantly lower than in the Normal Control group, with a mean difference of -1.400×10^9/L. Similarly, the Diabetes + Dioxin group had a significantly reduced sperm count, with a mean difference of -1.200×10^9/L. Both reductions were statistically significant, with p-values less than 0.01 and 0.05, respectively. In contrast, the Dioxin Only group did not show a statistically significant difference in sperm count compared to the control.

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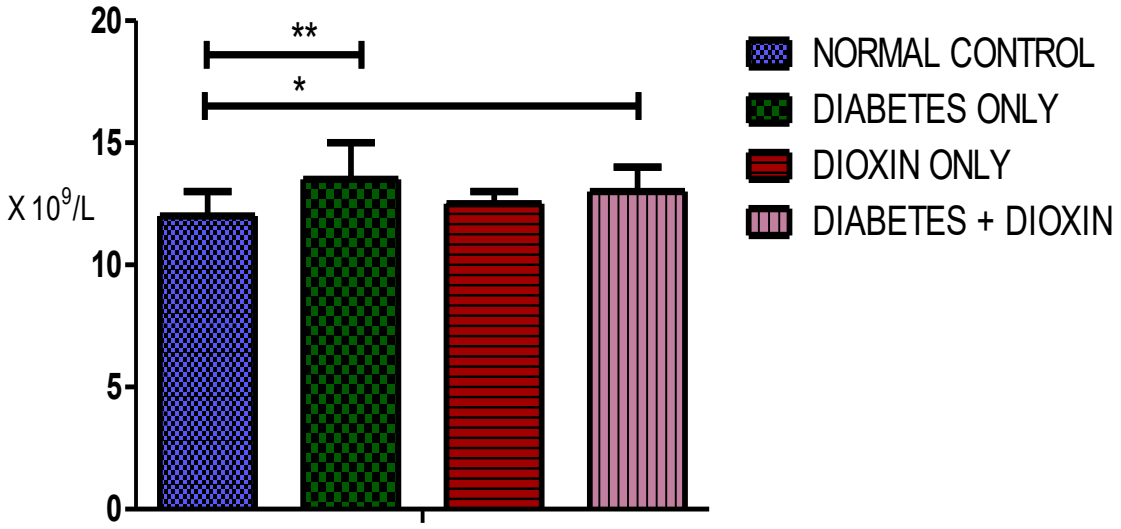

Figure 5: Bar graph showing sperm count (×10⁹/L) across experimental groups. Significant reductions were observed in the Diabetes Only and Diabetes + Dioxin groups compared to the Normal Control. Data are expressed as mean ± SD. *P < 0.05, **P < 0.01 vs. Normal Control (Dunnett's test).

Sperm viability

In the normal control group, sperm viability remained within the expected range. However, the diabetic-only group showed a noticeable reduction in viability compared to the control. A similar decline was observed in the Diabetes + Dioxin group, indicating that diabetes, whether alone or combined with dioxin exposure, negatively affects sperm viability. On the other hand, the Dioxin Only group exhibited an increase in sperm viability compared to the control group. Statistical analysis using one-way ANOVA confirmed that these differences were highly significant ($p < 0.0001$), with an F value of 113.7 and an R^2 of 0.9552, indicating that the treatment conditions could explain 95.52% of the variability in sperm viability. Bartlett's test for equal variances yielded a non-significant result ($p = 0.9874$), confirming the homogeneity of variances and validating the use of ANOVA. Further analysis with Dunnett's multiple comparison test showed that the Diabetic Only group had significantly lower sperm viability than the control group, with a mean difference of –2.640%. Similarly, the Diabetes + Dioxin group showed a significant reduction of –2.880% in viability. Interestingly, the Dioxin Only group had a significantly higher viability, with a mean increase of +4.952% compared to the Normal Control group. All these comparisons were statistically significant at $p < 0.001$.

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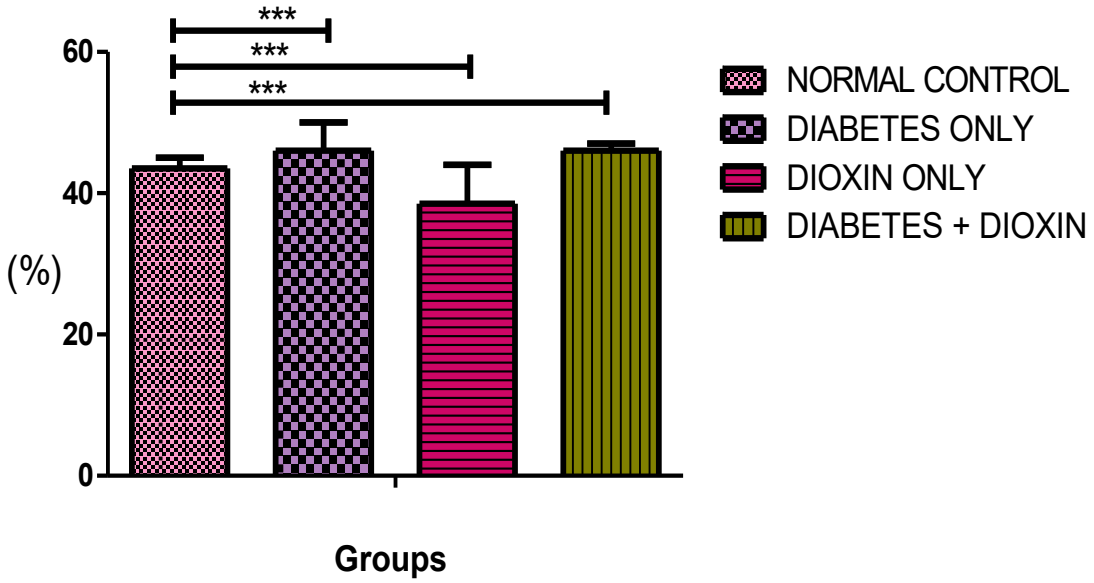

Figure 6: Bar graph showing sperm viability across experimental groups. Sperm viability was significantly reduced in the Diabetic Only and Diabetes + Dioxin groups compared to the Normal Control, while the Dioxin Only group showed a significant increase. Data are presented as mean ± SD. ***P < 0.001 vs. Normal Control (Dunnett's test).

Malondialdehyde (MDA)

The study assessed malondialdehyde (MDA) levels—an important indicator of lipid peroxidation and oxidative stress—in four distinct groups: Normal Control, Diabetic Only, Dioxin Only, and a combined Diabetic + Dioxin group. The results demonstrated notable differences in MDA concentrations among the groups. In the normal control group, MDA levels were the highest. In contrast, each of the experimental groups (Diabetic Only, Dioxin Only, and Diabetic + Dioxin) showed significantly lower levels of MDA. This was confirmed through one-way analysis of variance (ANOVA), which revealed a highly significant difference among the groups, with a p-value less than 0.0001 and an F-statistic of 221.8. The R-squared value of 0.9765 indicated that the observed group differences explained over 97% of the total variance in MDA levels. Additionally, Bartlett's test showed no significant difference in the variances between groups ($p = 0.7805$), validating the appropriateness of the ANOVA model. Post hoc analysis using Dunnett's multiple comparison test confirmed that all treated groups differed significantly from the normal control group. Specifically, MDA levels in the Diabetic Only group were significantly reduced, with a mean difference of 0.4800 µMg compared to the control. Similarly, the Dioxin Only group showed a significant reduction of 0.4140 µMg. Notably, the diabetic + dioxin group exhibited the greatest decline, with MDA levels lower by 0.6040 µMg relative to the control group. All comparisons were statistically significant with p-values less than 0.001.

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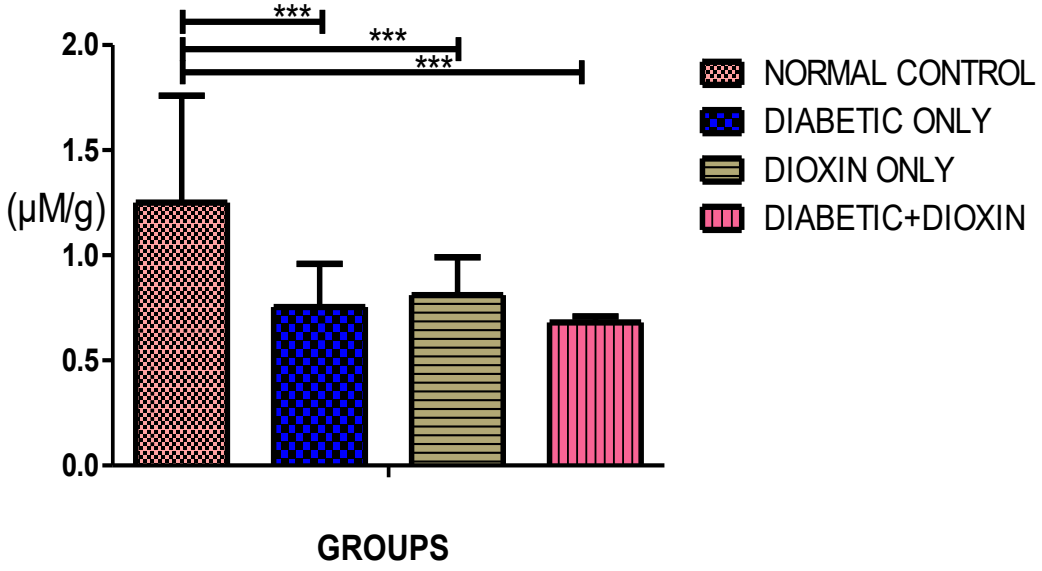

Figure 7: Bar graph showing malondialdehyde (MDA) levels (µMg) across experimental groups. The normal control group exhibited significantly higher MDA levels compared to the diabetic only, dioxin only, and diabetic + dioxin groups. Data are expressed as mean ± SD. ***P < 0.001 vs. Normal Control (Dunnett's test).

Superoxide dismutase (SOD)

The activity of superoxide dismutase (SOD) in testicular tissue varied significantly among the experimental groups, as illustrated in the attached bar graph. The normal control group displayed the highest mean SOD activity (approximately 5.4 U/mg protein), indicating robust antioxidant defence in healthy testes. In the diabetes-only (STZ) group, SOD activity was slightly reduced (around 4.8 U/mg protein) compared to the control; however, the difference was not statistically significant, as confirmed by Dunnett's multiple comparison test (mean difference = 0.07000; 95% CI: –0.3961 to 0.5361; $p > 0.05$). In contrast, both groups exposed to dioxin showed marked reductions in SOD activity. The dioxin-only group exhibited a significant decrease in SOD levels (approximately 1.4 U/mg protein) compared to the control group, with a mean difference of 3.714 (95% CI: 3.248 to 4.180; $p < 0.001$), demonstrating that dioxin exposure alone induces profound oxidative stress. Similarly, the STZ plus dioxin group had significantly lower SOD activity (around 2.6 U/mg protein) relative to the control, with a mean difference of 2.078 (95% CI: 1.612 to 2.544; $p < 0.001$), suggesting that combined exposure exacerbates oxidative imbalance beyond the effects of either insult alone. The one-way ANOVA revealed a highly significant difference among the four groups ($F(3,16) = 196.5$, $p < 0.0001$), with an R-squared value of 0.9736, indicating that nearly all the variation in SOD activity was accounted for by group differences. Bartlett's test for equal variances showed a significant result ($p = 0.0214$), suggesting heterogeneity of variances among the groups.

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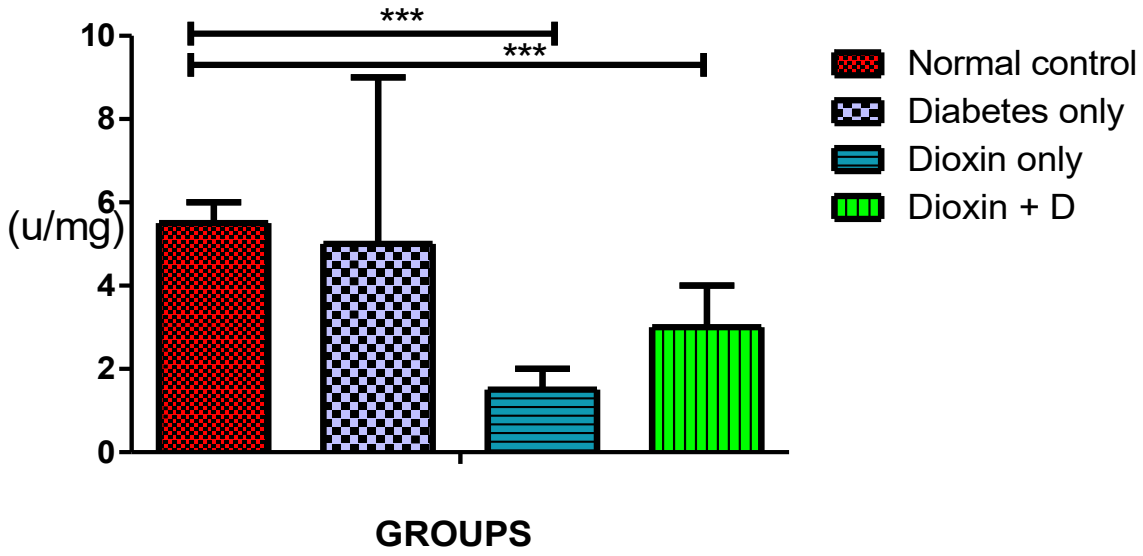

Figure 8: Bar graph showing testicular superoxide dismutase (SOD) activity (U/mg protein) across experimental groups. Data represent mean ± SEM (n=5). Both dioxin-treated groups (dioxin only and STZ + dioxin) show significantly reduced SOD activity compared to the normal control (***p < 0.001), indicating increased oxidative stress. Statistical analysis by one-way ANOVA with Dunnett's post-hoc test.

Histology (H&E)

Histological examination of the hematoxylin and eosin-stained testicular sections revealed marked differences among the experimental groups. In the normal control group (Slide A), the seminiferous tubules appeared well organized, round, and tightly packed. The germinal epithelium displayed a distinct stratification of spermatogenic cells at various stages of development, with lumens containing abundant mature spermatozoa. Interstitial spaces were normal in appearance, containing numerous Leydig cells, indicative of healthy and active spermatogenesis. In contrast, the STZ-only group (Slide B) demonstrated notable alterations. The seminiferous tubules were less densely packed and showed irregular contours. A reduction in the thickness of the germinal epithelium was observed in many tubules, suggesting impaired spermatogenesis. Additionally, several lumens were either empty or sparsely populated with spermatozoa, and the interstitial spaces appeared slightly expanded, potentially due to degeneration or oedema. These features are consistent with STZ-induced testicular damage, likely resulting from hyperglycemia-associated oxidative stress. The normal with the dioxin group (Slide C) exhibited mild histological disruptions. While the seminiferous tubules remained generally round, some showed disorganisation of the germinal epithelium with thinning of spermatogenic cell layers. The number of spermatozoa within the lumens was reduced compared to the control group, and the interstitial areas appeared slightly more prominent, although there were no apparent signs of inflammation. These findings suggest early signs of testicular toxicity attributable to the endocrine-disrupting and oxidative properties of dioxin. In the STZ plus dioxin group (Slide D), histological changes were most severe. Many seminiferous tubules exhibited pronounced disorganization and marked reduction in the thickness of the germinal epithelium. The lumens of several tubules were devoid of spermatozoa or contained cellular debris, and some tubules appeared shrunken or collapsed. The interstitial spaces were widened, with evidence of interstitial cell damage. These features indicate that combined exposure to STZ and dioxin led to synergistic testicular toxicity, resulting in severe spermatogenic arrest and extensive degeneration of testicular architecture.

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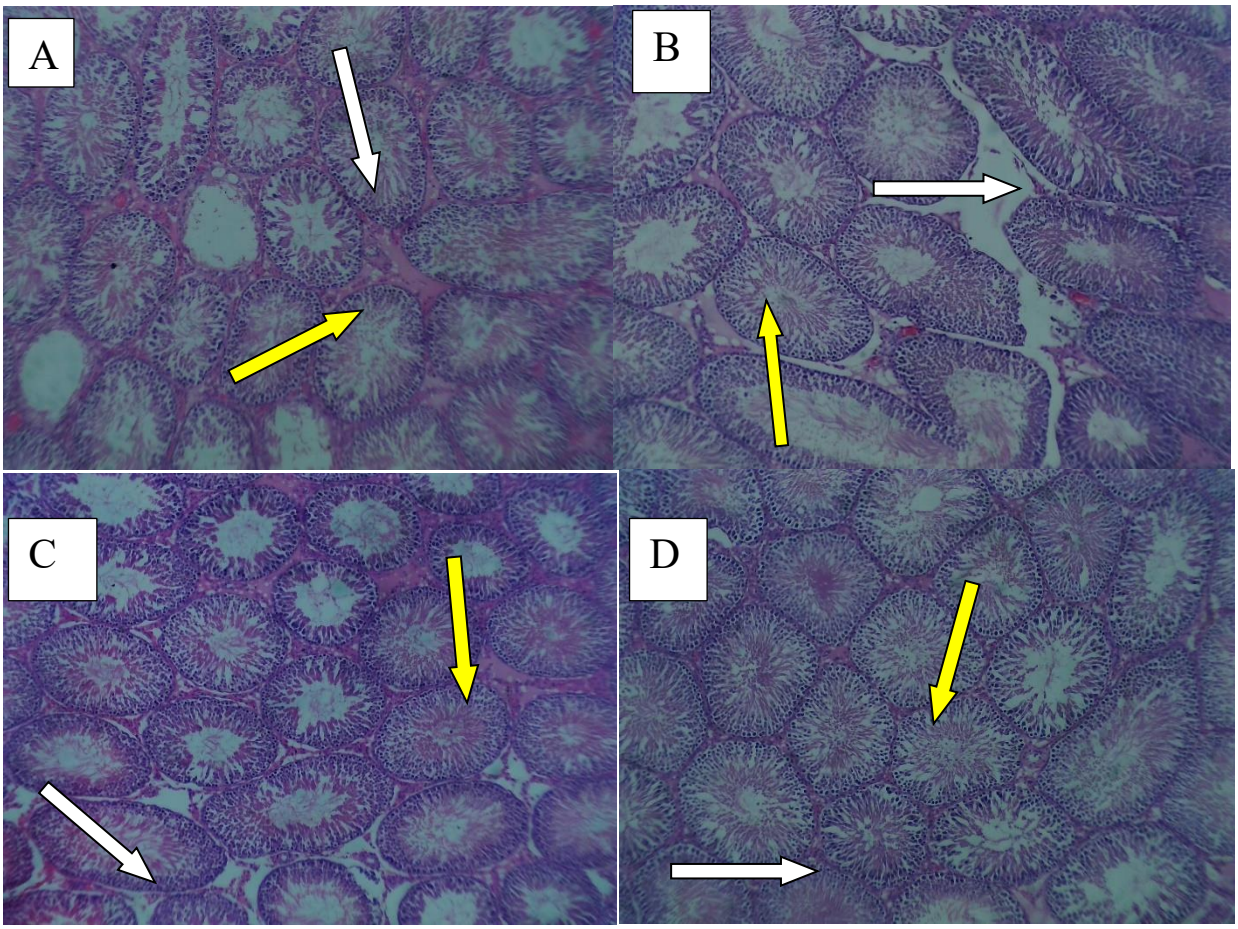

Plate 1: Photomicrographs of H&E-stained testicular sections (×400) from experimental groups. (A) Normal control showing intact seminiferous tubules with active spermatogenesis. (B) STZ-only group with reduced germinal epithelium and fewer luminal spermatozoa. (C) Normal + dioxin group exhibiting mild germinal disorganization. (D) STZ + dioxin group displaying severe tubular degeneration and spermatogenic arrest. White and yellow arrows indicate structural changes in the seminiferous epithelium.

Histology (Masson's trichrome)

Histological analysis of the Masson's trichrome-stained testicular sections revealed distinct patterns of tissue architecture and collagen deposition among the experimental groups. In the normal control group (Slide A), the seminiferous tubules appeared round and tightly packed and displayed an intact germinal epithelium with clear stratification of spermatogenic cells. Minimal blue staining was observed in the interstitial areas, indicating low collagen content and a normal extracellular matrix. The basement membranes of the tubules were thin and continuous, and the interstitial spaces contained numerous Leydig cells without evidence of fibrosis, consistent with a healthy testis and active spermatogenesis. In the STZ-only group (Slide B), notable histological alterations were evident. The seminiferous tubules appeared disorganised with reduced thickness of the germinal epithelium and occasional dilation of the lumens, many of which lacked mature spermatozoa. There was a marked increase in blue staining within the interstitial spaces and around the tubules, reflecting elevated collagen deposition and the development of interstitial fibrosis. Additionally, some tubules showed thickened basement membranes, consistent with early fibrotic changes

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associated with STZ-induced diabetic damage to the testis. The normal plus dioxin group (Slide C) demonstrated seminiferous tubules that were mainly preserved in overall structure but exhibited patchy thinning of the germinal epithelium and some tubular dilatation. There was a mild increase in blue staining within the interstitial areas, suggesting early signs of collagen accumulation and the initiation of a fibrotic response likely due to dioxin's toxic effects. Some tubules displayed early basement membrane thickening, although to a lesser extent than observed in the STZ-only group. The STZ plus dioxin group (Slide D) revealed the most severe histological damage. Many seminiferous tubules were markedly degenerated with pronounced disorganisation and extensive loss of the germinal epithelium. Large, empty lumens devoid of spermatogenic cells were typical. The interstitial tissue exhibited intense blue staining, indicating widespread collagen deposition and advanced interstitial fibrosis. Thickening of the basement membranes was more extensive than in either of the individual treatment groups, highlighting the synergistic effect of combined STZ and dioxin exposure in promoting profound testicular degeneration and fibrotic remodelling.

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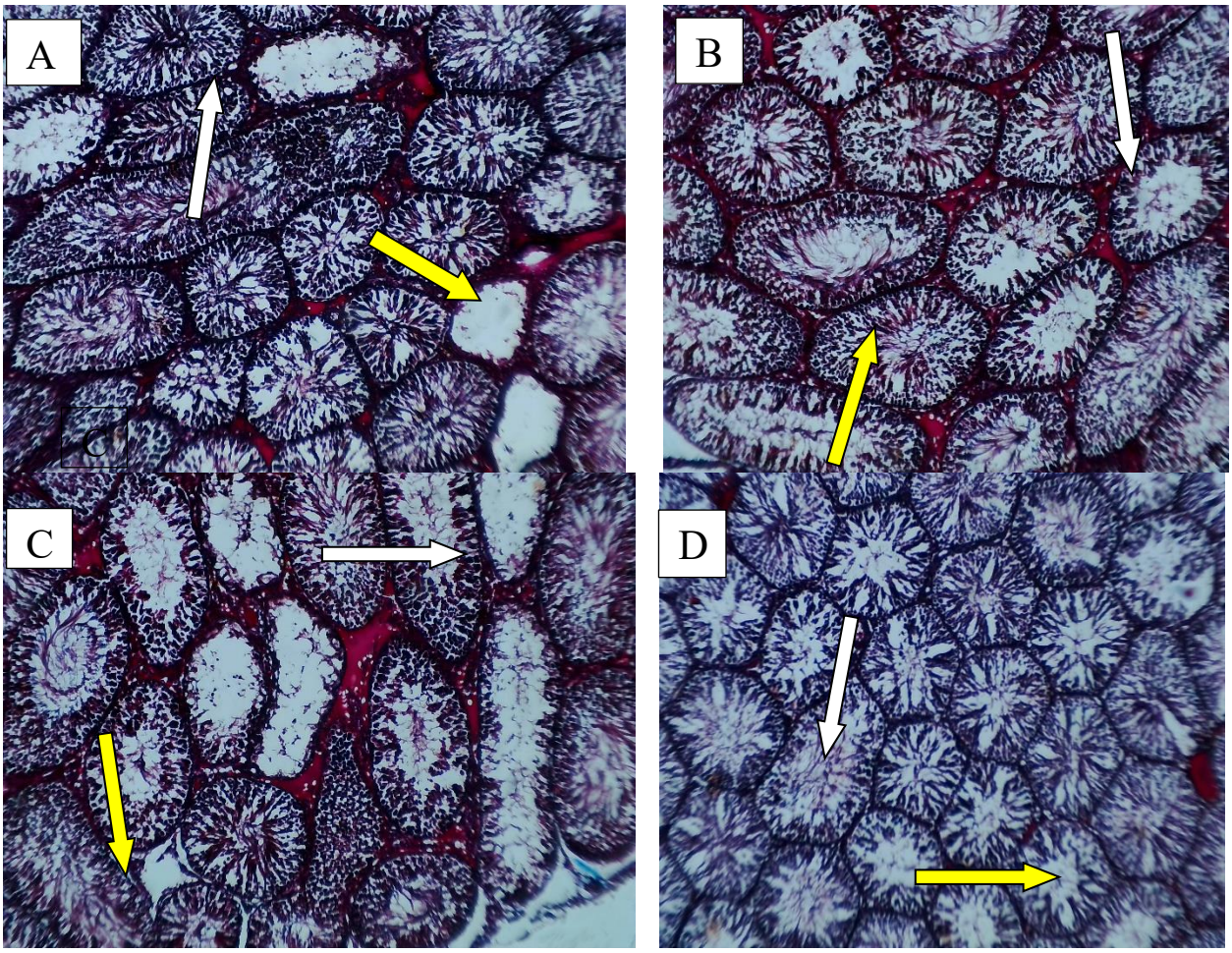

Plate 2: Photomicrographs of Masson's trichrome-stained testicular sections (×400). (A) Normal control showing intact seminiferous tubules with minimal interstitial collagen. (B) STZ-only group displaying tubular disorganization and increased interstitial fibrosis. (C) Normal + dioxin group showing mild epithelial thinning and early collagen deposition. (D) STZ + dioxin group exhibiting severe tubular degeneration and extensive interstitial fibrosis.

Discussion

This study provides compelling evidence that both hyperglycemia (induced by STZ) and exposure to dioxin independently impair testicular structure and function and that their combination exerts a synergistic, deleterious effect on male reproductive health. It demonstrated significant alterations in metabolic, hormonal, and spermatogenic parameters, supported by profound histopathological changes.

Glycemic control and body weight: Although group means for fasting blood sugar (FBS) did not differ significantly across all time points, the steeper week-4/5 rise in the diabetic and diabetic+dioxin groups mirrors reports that dioxin aggravates glucose intolerance and insulin resistance by impairing β-cell function and peripheral insulin signaling via AhR-dependent transcriptional reprogramming and hepatic enzyme induction (Kishi et al., 2025; Novelli et al., 2021). Diabetes alone increased body weight early, consistent with STZ-linked hyperphagia. In

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contrast, dioxin—alone or with diabetes—blunted weight gain, aligning with studies showing dioxin's catabolic actions, gut barrier/inflammatory effects, and endocrine interference that reduce nutrient utilisation (Paul *et al.,* 2022; Johnson *et al.,* 2020).

Hormonal profile: A rise in testosterone was observed in diabetes alone, but suppression with dioxin and with the combined treatment was observed. Transient hyperandrogenism under acute/early diabetic stress has been attributed to compensatory Leydig activation or altered sex-hormone binding dynamics; however, dioxin is widely documented to repress Leydig steroidogenesis by AhR activation, down-regulating StAR, CYP11A1, and 17β-HSD and perturbing hypothalamic–pituitary–gonadal signalling (Gang *et al.,* 2022). Combined-exposure data indicate that dioxin's anti-steroidogenic action can override diabetes-related compensations, consistent with endocrine-disruptor literature.

Spermatogenesis and semen quality: The sharp declines in motility, viability, and count in diabetes and diabetes+dioxin groups agree with clinical and experimental evidence that hyperglycemia elevates seminal ROS, damages mitochondrial bioenergetics, and fragments nuclear/mtDNA (Omolaoye *et al.,* 2021; Barkabi-Zanjani *et al.,* 2020; Darmishonnejad *et al.,* 2024). The further decrement with co-exposure suggests additive/synergistic hits on mitochondrial function and axonemal integrity. Intriguingly, dioxin alone increased motility/viability in our setting. Similar paradoxes have been attributed to dose-, timing-, or hormesis-like responses where low-level AhR activation transiently up-regulates stress-response or detox genes that can momentarily favour sperm function. Because most reports link TCDD to poorer semen quality, we interpret this as a context-specific response that warrants dose–response and time-course resolution.

Oxidative stress and antioxidant defences: Markedly reduced SOD activity in dioxin and combined groups, with altered MDA, indicates redox disequilibrium. Prior work shows that both diabetes and TCDD heighten ROS through mitochondrial electron-transport leakage, NADPH oxidase activation, and cytochrome P450 induction, while simultaneously depressing enzymatic defenses (Wang *et al.,* 2022; Darmishonnejad *et al.,* 2024). Reduced SOD in our dioxin-exposed testes is congruent with this mechanism and provides a plausible driver for the observed DNA damage, impaired steroidogenesis, and defective spermatogenesis.

Testicular architecture and fibrosis: H&E and Masson's trichrome findings—germinal epithelium thinning, tubular disorganisation, luminal depletion, and extensive interstitial collagen—mirror diabetes-associated BTB disruption and dioxin-triggered profibrotic signalling (e.g., TGF-β/SMAD), culminating in extracellular-matrix deposition and seminiferous dysfunction. The most severe remodelling in the combined group supports a model in which hyperglycemia-induced oxidative/inflammatory priming sensitises the testis to AhR-mediated fibrogenesis. Taken together, the outcome of this study aligns with reports linking hyperglycemia to oxidative sperm damage and with robust evidence that TCDD, via AhR, is anti-steroidogenic and pro-fibrotic (Abbasi et al., 2020; Leisegang et al., 2017; Gang et al., 2022). it extends this literature by demonstrating that concurrent metabolic and toxicant stressors produce a compound phenotype—greater endocrine suppression, deeper antioxidant collapse, and more advanced histological injury—than either insult alone.

Potential mechanisms behind observed effects include: (1) Diabetes elevates ROS and perturbs Sertoli glucose handling (GLUT-mediated transport/lactate provision), compromising germ-cell support; (2) dioxin activates AhR, represses steroidogenic genes, interferes with ER/AR signalling, and induces inflammatory/pro-fibrotic programs; (3) co-exposure amplifies mitochondrial dysfunction and cytokine signalling (e.g., TNF-α, IL-6, TGF-β), accelerates BTB failure, and drives

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collagen deposition—ultimately reducing testosterone and degrading spermatogenesis. Overall, the present findings are concordant with—and mechanistically anchored in—the endocrine-disruption and diabetic-infertility literature, while highlighting the heightened reproductive risk when environmental and metabolic stressors intersect. Future work should quantify steroidogenic gene expression (StAR, CYP11A1, 17β-HSD), assess apoptosis markers (Bax/Bcl-2, caspase-3), and perform dose–time modelling of TCDD to clarify the hormetic window suggested by the motility result.

Conclusion and recommendations

Given the global rise in diabetes and the persistence of dioxins in food and the environment, these results reinforce the need for integrated strategies that address both metabolic control and environmental safety. Strengthening regulatory measures to limit dioxin contamination, alongside optimising glycemic management, may represent complementary avenues for reducing reproductive risk. Clinically, the data suggest that men with diabetes may constitute a vulnerable subgroup in whom environmental exposures could have disproportionately harmful reproductive consequences.

Further research is warranted to elucidate the molecular pathways involved in this synergism, particularly the roles of inflammatory cytokines, mitochondrial dysfunction, and epigenetic modifications. Additionally, these findings emphasise the need for strategies to mitigate combined environmental and metabolic risk factors in male reproductive health.

Professional implications of the study

The findings indicate that while diabetes and dioxin exposure showed trends toward elevated fasting blood sugar levels, the absence of statistically significant differences suggests that short-term exposure or interaction may not exert a pronounced effect on glycemic regulation. For professionals in biomedical research and clinical practice, this highlights the need for extended longitudinal studies and larger sample sizes to fully evaluate the metabolic consequences of combined toxicological and pathological conditions. Such insights are essential for informing risk assessments, developing targeted interventions, and guiding policies on environmental exposures in diabetic populations.

Limitations to the study

This study was limited by the relatively small sample size, which may have reduced the statistical power to detect subtle differences in fasting blood sugar levels among groups. In addition, the significant variance observed between groups suggests heterogeneity in response, which could have influenced the outcome. The study was also restricted to a short experimental period, making it difficult to fully capture the long-term effects of diabetes and dioxin exposure on glucose metabolism. Future studies with larger cohorts, extended duration, and tighter control of confounding factors are recommended to strengthen the findings.

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Ethical approval

Not applicable

Informed consent

Not applicable

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