

OXIDATIVE STRESS MITIGATION AND REPRODUCTIVE FUNCTION RESTORATION VIA COSTUS AFER IN DIABETIC MICE

OWO, G. J.^{1*} – ENYINDAH, K. C.¹

¹ Faculty of Natural and Applied Sciences, Ignatius Ajuru University of Education, Rivers State, Nigeria.

*Corresponding author
e-mail: james.owo[at]iaue.edu.ng

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Abstract. Diabetes mellitus has consistently been a chronic metabolic disorder that is strongly linked with disorders of the male reproductive system. This disorder is commonly due to oxidative stress-induced testicular damage. Persistent hyperglycaemia has been well documented as one factor that promotes the production of excessive reactive oxygen species (ROS) that leads to lipid peroxidation, antioxidant depletion, and impaired spermatogenesis. This study examined the oxidative stress-mediated reproductive dysfunction and the modulatory role of hydromethanolic leaf extract of *Costus afer* in streptozotocin-induced diabetic mice. Thirty (30) adult male Swiss albino mice were allocated into five groups (n = 6) using random sampling technique. Diabetes was induced intraperitoneally using streptozotocin (50 mg/kg). Diabetic rats were orally given either metformin (100 mg/kg) or hydromethanolic leaf extract of *C. afer* (200 and 400 mg/kg) for a duration of 28 days. Fasting blood glucose was monitored, while testicular malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) were assessed spectrophotometrically. Sperm count, motility, morphology, serum testosterone, and histopathological changes were evaluated. Streptozotocin-induced diabetes resulted in persistent hyperglycemia, significantly elevated MDA levels, reduced antioxidant enzyme activities, decreased sperm count and motility, increased abnormal sperm morphology, and reduced testosterone concentrations ($p < 0.05$). Treatment with hydromethanolic leaf extract of *C. afer* significantly reduced fasting blood glucose, attenuated lipid peroxidation, restored antioxidant enzyme activities, improved sperm quality, increased testosterone levels, and ameliorated histopathological alterations in a dose-dependent manner ($p < 0.05$), with the 400 mg/kg dose demonstrating effects comparable to metformin. Hydromethanolic leaf extract of *C. afer* effectively relieves oxidative stress-mediated reproductive dysfunction in diabetic mice. The findings provide experimental evidence supporting its antioxidant-driven protective role and highlight its potential as an adjunct therapeutic candidate in the management of diabetes-associated male infertility.

Keywords: diabetes mellitus, oxidative stress, reproductive dysfunction, costus afer, streptozotocin, antioxidant enzymes

Introduction

Diabetes Mellitus (DM) is a chronic endocrine and metabolic disorder characterised by persistent hyperglycemia resulting from impaired insulin secretion (Owo, 2026; Owo and Beresford, 2020), insulin resistance (Obadiah et al., 2025), or both (Ojo et al., 2023). Beyond its well-established vascular and metabolic complications, the disease has been progressively linked to male reproductive dysfunction (He et al., 2021), manifesting as reduced sperm count and motility, increased abnormal sperm morphology, and decreased testosterone levels (Badejogbin et al., 2025; He et al., 2021; Barkabi-Zanjani et al., 2020). According to Huang et al. (2024) and Güleş (2023), chronic hyperglycaemia negatively affects testicular morphology and endocrine function, hence leads to subfertility and infertility in distressed males. The main

mechanism that brings about diabetes-associated reproductive impairment is oxidative stress (Dena et al., 2025; He et al., 2021). In this mechanism, sustained hyperglycaemia enhances the generation of reactive oxygen species (ROS) via glucose auto-oxidation (Qasim et al., 2022), mitochondrial dysfunction (Black, 2022; Deragon et al., 2020), and progressive glycation end-product formation (Nakamura and Kawaharada, 2021). Here, extreme ROS production suppresses endogenous antioxidant defenses, leading to lipid peroxidation, protein oxidation, and DNA damage within testicular tissue (Rotimi et al., 2024), hence the reason for the elevation of malondialdehyde (MDA) levels and the reduction in the levels of antioxidant enzymes, which are antioxidant biomarkers in diabetic conditions (Goycheva et al., 2023; Kwong-Han et al., 2022). Also, due to the high concentration of polyunsaturated fatty acids and severe metabolic activities in the testes, the tissue is substantially prone to oxidative stress. This may be because of excess reactive oxygen species inducing lipid peroxidation and cellular damage within the seminiferous tubules. Consequently, oxidative injuries have been known to disrupt spermatogenesis and impair the functional activity of Leydig cells (Ju et al., 2025).

Medicinal plants with antioxidant and hypoglycaemic activities have gained increasing attention as potential adjunct therapies (Kpomah and Owo, 2024a; Ezekwe et al. 2021). *Costus afer* is a well-known medicinal plant widely used in African ethnomedicine (Boison et al., 2019). It has confirmed antidiabetic (Laide et al., 2025), anti-inflammatory (Kpomah and Owo, 2024b), and antioxidant activities (Oyetayo et al., 2025a). The extracts of the different parts of *C. afer* have been explored and shown to have several active medicinal compounds in them. For instance, the flavonoids and phenolic compounds in them have been revealed to scavenge free radicals (Ekor et al., 2024), boost the activities of endogenous antioxidant enzymes (Oyetayo et al., 2025b), and reduce the effects of lipid peroxidation (Ndoni et al., 2017). Even though synthetic antidiabetic agents like metformin have been produced to effectively improve glucose homeostasis, their ability to completely reverse oxidative damage in reproductive tissues remains limited (Chaudhary and Kulkarni, 2024; Dutta et al., 2023; Lin et al., 2022), calling for complementary approaches that focus on both hyperglycemia and redox imbalance. Therefore, this study aimed to investigate the modulatory effects of hydromethanolic leaf extract of *C. afer* on fasting blood glucose, oxidative stress biomarkers, and reproductive parameters in streptozotocin-induced diabetic mice.

Materials and Methods

This study employed a randomised, controlled experimental design to evaluate the modulatory effect of hydromethanolic leaf extract of *C. afer* on oxidative stress-mediated reproductive dysfunction in streptozotocin-induced diabetic mice. The study duration was 28 days following diabetes induction. All experimental procedures were conducted in accordance with institutional guidelines for the care and use of laboratory animals and were approved by the Research Ethics Committee of Rivers State University (Approval No: RSU/FBMS/REC/26/047). Thirty (30) adult male albino mice (8–10 weeks old), of average weight of 160 g, were obtained from the animal facility of Rivers State University. The animals were housed in standard polypropylene cages under controlled environmental conditions (12-hour light/dark cycle, temperature $22 \pm 2^\circ\text{C}$, relative humidity 50–60%) and had free access to standard pellet diet and water ad libitum. The animals were acclimatised for two weeks prior to experimentation. Fresh leaves of *C. afer* were collected, authenticated by a plant taxonomist in the Department

of Plant Science and Biotechnology, Rivers State University, Port Harcourt, Rivers State, Nigeria, and air-dried at room temperature. The dried leaves were pulverised into fine powder. Five hundred grams (500 g) of the powdered sample were macerated in 80% methanol (hydromethanol) for 72 hours with intermittent agitation. The mixture was filtered using Whatman No. 1 filter paper and concentrated under reduced pressure using a rotary evaporator at 40°C. The extract was further dried in a water bath to obtain a semi-solid residue and stored at 4°C until use. Experimental diabetes was induced by a single intraperitoneal injection of streptozotocin (STZ) at a dose of 50 mg/kg body weight, freshly prepared in 0.1 M cold citrate buffer (pH 4.5), following an overnight fast. To prevent initial hypoglycemic shock, mice were provided 5% glucose solution for 24 hours post-injection. After 72 hours, fasting blood glucose (FBG) levels were measured using a glucometer via tail vein puncture. Mice with FBG \geq 250 mg/dL were considered diabetic and included in the study.

Eligible mice were randomly assigned using a simple randomisation technique (computer-generated random allocation) into five groups (n = 6 per group): (1) Group I (Environmental control): Non-diabetic mice administered distilled water. (2) Group II (Negative control): STZ-induced diabetic mice administered distilled water. (3) Group III: Diabetic mice treated with 200 mg/kg hydromethanolic leaf extract of *C. afer*. (4) Group IV: Diabetic mice treated with 400 mg/kg hydromethanolic leaf extract of *C. afer*. (5) Group V (Positive control): Diabetic mice treated with metformin (100 mg/kg). All treatments were administered orally once daily for 28 consecutive days. Fasting blood glucose levels were assessed at baseline, 72 hours after STZ administration to confirm the induction of diabetes, and subsequently on a weekly basis using a digital glucometer. Blood samples were collected from the tail vein following an overnight fast. At the end of the treatment period, the mice were fasted overnight and euthanised under mild anesthesia. Blood samples were collected via cardiac puncture for serum separation. The testes were excised, blotted dry, and weighed. Testicular tissues were later homogenised in phosphate-buffered saline and centrifuged. The supernatant was used for biochemical analyses. Malondialdehyde (MDA) levels (in mmol/mg protein) were determined as an index of lipid peroxidation using the thiobarbituric acid reactive substances (TBARS) method described by Okari et al. (2025), while superoxide dismutase (SOD) activity (in U/mg protein) was assessed based on inhibition of epinephrine auto-oxidation according to the method of Misra and Fridovich (1972). The catalase (CAT) activity (in U/mg protein) was measured using a spectrophotometric method of Uro-Chukwu et al. (2024), whereas glutathione peroxidase (GPx) activity (in U/mg protein) was determined using the coupled enzyme method of Rotruck et al. (1973). All assays were performed according to established protocols.

Sperm analysis was performed using standard laboratory procedures for rodent semen evaluation. Briefly, the cauda epididymis was excised and immediately placed in 1 mL of pre-warmed normal saline (0.9% NaCl) at 37°C. The tissue was minced with sterile scissors and incubated for 10–15 minutes at 37°C to allow adequate sperm dispersion into the medium. The resulting suspension was gently mixed prior to assessment. (1) Sperm Count: Sperm concentration was determined using an improved Neubauer hemocytometer following appropriate dilution of the sperm suspension with normal saline. A drop of the diluted sample was loaded into the counting chamber, and sperm cells were counted under a light microscope at \times 400 magnification. The total sperm count was calculated and expressed as $\times 10^6$ sperm/mL. (2) Sperm Motility: Sperm motility was evaluated by placing a drop of freshly prepared sperm suspension

on a clean glass slide, covered with a coverslip, and examined microscopically at $\times 400$ magnification. At least five randomly selected fields were observed per sample. Motile and non-motile spermatozoa were counted, and motility was expressed as the percentage of progressively motile sperm cells. (3) Sperm Morphology: Sperm morphology was assessed using eosin–nigrosin staining. A thin smear of sperm suspension was prepared on a clean slide, air-dried, and stained with eosin–nigrosin stain. The slide was examined under oil immersion ($\times 1000$ magnification). A minimum of 200 spermatozoa per sample were evaluated for structural abnormalities involving the head, midpiece, and tail. Results were expressed as the percentage of morphologically normal spermatozoa. Serum testosterone levels were measured using the Testosterone ELISA Kit (Cayman Chemical, Ann Arbor, MI, USA; Cat. No. 582701) according to the manufacturer’s instructions. After the animals were sacrificed; the testicular tissues of all the rats in the five groups were harvested and examined histologically (Stain: haematoxylin and eosin; magnification $\times 200$). Photomicrographs of different testicular sections were taken. Data were expressed as mean \pm standard deviation (SD). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey’s post hoc test for multiple comparisons. Statistical significance was set at $P < 0.05$.

Results and Discussion

Effect on fasting blood glucose

As presented in *Table 1*, baseline fasting blood glucose (FBG) levels did not differ significantly among the groups ($p > 0.05$). Seventy-two hours after streptozotocin (STZ) administration, diabetic groups showed a significant elevation in FBS compared with the normal control ($p < 0.05$), confirming successful induction of diabetes. At Weeks 2 and 4, the diabetic control group maintained significantly higher FBG levels relative to the normal control ($p < 0.05$). However, treatment with hydromethanolic leaf extract of *C. afer* resulted in a significant and dose-dependent reduction in FBS compared with the diabetic control ($p < 0.05$). The 400 mg/kg dose demonstrated greater glycemic improvement by Week 4, with values comparable to those observed in the metformin-treated group. In the effect of *C. afer* extract on fasting blood glucose, treatment with *Costus afer* extract produced a significant, dose-dependent reduction in fasting blood glucose (FBG) as shown in *Table 1*, clearly demonstrating its antihyperglycemic efficacy against streptozotocin-induced diabetes. Similar findings have been reported in previous studies, where *C. afer* extracts significantly lowered blood glucose levels in diabetic models, with higher doses producing more pronounced effects (Ezeigwe et al., 2020; Ezejiofor et al., 2015).

Table 1. Fasting blood glucose levels (mg/dL).

Group	Baseline	Post-STZ (72h)	Day 28
Normal Control	92.4 \pm 3.1	94.6 \pm 2.8	95.2 \pm 3.0
Diabetic Control	93.7 \pm 2.9	312.5 \pm 8.6*	328.4 \pm 10.2*
Metformin	94.1 \pm 3.0	305.8 \pm 7.9*	118.6 \pm 4.5**
<i>C. afer</i> 200 mg/kg	92.9 \pm 3.2	308.4 \pm 8.3*	156.7 \pm 6.2**
<i>C. afer</i> 400 mg/kg	93.3 \pm 2.7	310.2 \pm 8.1*	124.3 \pm 5.1**

Note: * $p < 0.05$ vs Normal Control; ** $p < 0.05$ vs Diabetic Control.

Effect on oxidative stress biomarkers

The effect of *C. afer* on testicular oxidative stress markers is shown in *Table 2*. Diabetic control mice exhibited a significant increase in malondialdehyde (MDA) levels compared with the normal control ($p < 0.05$), indicating enhanced lipid peroxidation. In contrast, activities of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) were significantly reduced in the diabetic control group ($p < 0.05$). Treatment with hydromethanolic leaf extract significantly decreased MDA levels and significantly increased SOD, CAT, and GPx activities compared with the untreated diabetic group ($p < 0.05$). The improvement was dose-dependent, with the 400 mg/kg group showing antioxidant enzyme activities approaching those of the normal and metformin-treated groups. In effect of *C. afer* extract on oxidative stress biomarkers, the extract significantly decreased malondialdehyde (MDA) levels while restoring the activities of key antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) in dose-dependent manner (*Table 2*). This indicates a reversal of oxidative imbalance induced by diabetes. The antioxidant effect of *C. afer* may likely be mediated by its phytochemical constituents, particularly flavonoids and phenolics, which scavenge free radicals and enhance endogenous defense systems (Anyanwu et al., 2025; Bennici et al., 2024; Sun and Shahrajabian, 2023).

Table 2. Oxidative stress biomarkers in testicular tissue.

Group	MDA (nmol/mg protein)	SOD (U/mg protein)	CAT (U/mg protein)	GPx (U/mg protein)
Normal Control	2.15 ± 0.12	8.45 ± 0.30	52.3 ± 1.5	14.6 ± 0.5
Diabetic Control	5.92 ± 0.25*	4.12 ± 0.20*	28.7 ± 1.2*	7.2 ± 0.4*
Metformin	2.80 ± 0.18**	7.60 ± 0.28**	48.5 ± 1.3**	13.2 ± 0.6**
<i>C. afer</i> 200 mg/kg	3.45 ± 0.20**	6.85 ± 0.25**	44.2 ± 1.1**	11.9 ± 0.5**
<i>C. afer</i> 400 mg/kg	2.60 ± 0.15**	7.90 ± 0.32**	50.8 ± 1.4**	13.8 ± 0.5**

Note: * $p < 0.05$ vs Normal Control; ** $p < 0.05$ vs Diabetic Control.

Effect on sperm parameters

As shown in *Table 3*, the STZ-induced diabetes led to significant reductions in sperm parameters (sperm count, motility, and morphology) when compared with the normal control ($p < 0.05$). The oral administration of hydromethanolic leaf extract of *C. afer* significantly improved all sperm parameters relative to the diabetic control group ($p < 0.05$). The higher dose (400 mg/kg) produced more pronounced improvements, closely aligning with the metformin-treated group. In effect of *C. afer* extract on sperm parameters, administration of *C. afer* extract significantly improved sperm count, motility, morphology, and viability (*Table 3*), demonstrating its protective role against diabetes-induced reproductive toxicity. Oxidative stress is known to impair spermatogenesis by damaging germ cells (Guo et al., 2021; Aitken, 2020; Yang et al., 2019), disrupting membrane integrity, and reducing mitochondrial ATP production required for sperm motility (Chen et al., 2022; Castellini et al., 2021; Zhu et al., 2019). The observed improvement suggests that the extract possesses antioxidants which preserve testicular function by mitigating oxidative damage and maintaining cellular homeostasis, thereby supporting normal spermatogenic processes (Asadi et al., 2017).

Table 3. Effect of extract of *C. afer* on sperm parameters.

Group	Sperm Count ($\times 10^6/\text{mL}$)	Motility (%)	Abnormal Morphology (%)
Normal Control	78.4 \pm 3.2	82.5 \pm 2.1	5.2 \pm 0.6
Diabetic Control	42.6 \pm 2.8*	48.7 \pm 1.9*	18.4 \pm 1.1*
Metformin	71.3 \pm 3.0**	76.2 \pm 2.0**	7.4 \pm 0.8**
<i>C. afer</i> 200 mg/kg	64.8 \pm 2.7**	69.5 \pm 1.8**	9.6 \pm 0.7**
<i>C. afer</i> 400 mg/kg	74.5 \pm 2.9**	80.4 \pm 2.2**	6.1 \pm 0.6**

Note: * $p < 0.05$ vs Normal Control; ** $p < 0.05$ vs Diabetic Control.

Effect on serum testosterone

Serum testosterone levels were significantly reduced in the diabetic control group compared with the normal control ($p < 0.05$). Treatment with both doses of *Costus afer* extract significantly increased testosterone levels relative to the untreated diabetic group ($p < 0.05$), with greater restoration observed at 400 mg/kg. These findings are presented in Table 4. In effect of *costus afer* extract on serum testosterone, the findings presented in Table 4 showed that the extract significantly restored serum testosterone levels, indicating improved Leydig cell function and steroidogenic activity. Diabetes-associated oxidative stress disrupts testosterone biosynthesis by impairing key enzymatic pathways (Jangid et al., 2025; He et al., 2021; Shaikh et al., 2016). By reducing oxidative stress, *C. afer* may have likely enhanced the functional integrity of Leydig cells, leading to increased androgen production (Kaltsas, 2023; Monageng et al., 2023). This restoration of testosterone may have contributed synergistically to the improvement in sperm parameters, given its critical role in spermatogenesis and male reproductive health (Ezejiolor and Orisakwe, 2019; Arhoghro and Sule, 2017).

Table 4. Effect of extract of *C. afer* on serum testosterone levels.

Group	Testosterone (ng/mL)
Normal Control	3.85 \pm 0.20
Diabetic Control	1.72 \pm 0.15*
Metformin	3.40 \pm 0.18**
<i>C. afer</i> 200 mg/kg	3.02 \pm 0.17**
<i>C. afer</i> 400 mg/kg	3.65 \pm 0.19**

Note: * $p < 0.05$ vs Normal Control; ** $p < 0.05$ vs Diabetic Control.

Histopathological changes

Group I (Environmental control): Well-organized seminiferous tubules with complete spermatogenic series; abundant spermatozoa (S) in lumen; normal Leydig cells (L) in interstitium. Group II (Negative control): Marked degeneration of seminiferous tubules; thinning of germinal epithelium (thin arrows); vacuolation (thick arrows); reduced/absent spermatozoa; interstitial inflammation. Group III (*C. afer* 200 mg/kg): Partial restoration of seminiferous tubules; moderate spermatogenic activity; some spermatozoa in lumen; occasional vacuoles (arrow); partial Leydig cell recovery. Group IV (*C. afer* 400 mg/kg): Seminiferous tubules nearly normal; complete spermatogenic series; abundant spermatozoa in lumen; normal Leydig cells. Group V (Positive control): Substantial improvement; well-organized tubules with active spermatogenesis; mild residual changes; preserved Leydig cells (Figure 1). The effect of *costus afer* extract on histopathological changes, in the diabetic control group, severe deterioration of seminiferous tubules, reduction of germinal epithelium, and huge

vacuolation were seen. These changes correlated strongly with the elevated malondialdehyde (MDA) levels and reduced antioxidant enzyme activities, confirming increased oxidative stress. This is consistent with several studies that reported that diabetes-induced oxidative stress alters testicular architecture and impairs spermatogenesis through lipid peroxidation and cellular apoptosis (Guo et al., 2021; Aitken, 2020; Yang et al., 2019). The structural injury to germ cells clarifies the significant decline in sperm count, motility, and morphology, while the reduced population of Leydig cells aligns with the observed decrease in serum testosterone levels. Similarly, previous studies like those of He et al. (2021) and Shaikh et al. (2016) have clarified that oxidative stress subdues the activities of steroidogenic enzymes, leading to lowered testosterone production.

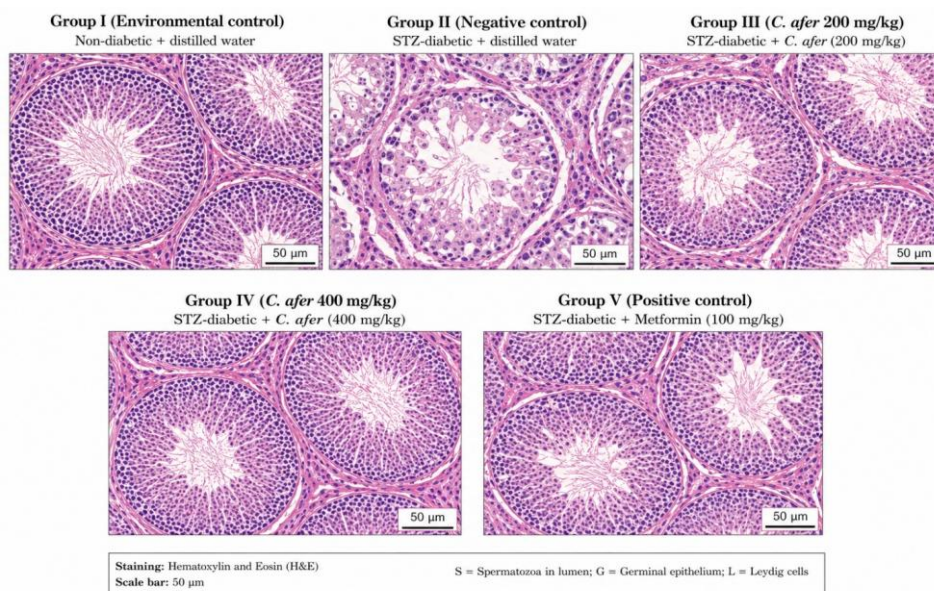


Figure 1. Representative photomicrographs of testicular sections from experimental rats showing the effects of hydromethanolic leaf extract across groups (H&E Staining).

The treatment given to the rats with hydromethanolic leaf extract at 200 mg/kg caused a moderate restoration of seminiferous tubule architecture and partial regeneration of spermatogenic cells. This structural improvement is consistent with the observed reduction in MDA levels and enhancement of antioxidant enzyme activities. Comparable findings have been reported in plant-based antioxidant therapies, where partial restoration of testicular histology corresponds with improved redox balance and spermatogenic activity (Castellini et al., 2021; Asadi et al., 2017). The partial recovery of testicular architecture supports the corresponding improvements in sperm parameters and testosterone levels (Askar et al., 2025), although not fully restored, indicating a dose-limited therapeutic effect. At 400 mg/kg, the extract produced an almost complete restitution of testicular histoarchitecture, with well-organised seminiferous tubules, active spermatogenesis, and preserved Leydig cells. These histological findings strictly align with the noteworthy normalisation of oxidative stress biomarkers, pointing towards effective lowering of lipid peroxidation and boosting of endogenous antioxidant systems. Similar dose-dependent protective effects have been documented for phytochemical-rich extracts, particularly those containing flavonoids and phenolics, which enhance antioxidant enzyme expression and protect germ cells from oxidative

injury (Bennici et al., 2024; Sun and Shahrajabian, 2023). The abundance of spermatozoa within the tubules directly validates the marked improvements in sperm count, motility, and morphology (Ogunlade et al., 2022). Furthermore, the preservation of Leydig cells validates the restoration of testosterone levels, confirming the recovery of steroidogenic function. This supports findings that antioxidant-mediated protection of Leydig cells restores the production of androgens and helps in spermatogenesis (Kaltsas, 2023; Monageng et al., 2023). Similarly, the metformin-treated group displayed substantial histological recovery, which is in line with its ability to lower oxidative stress and enhance blood sugar control. Previous studies have shown that metformin restores testicular damage by reducing hyperglycaemia-induced ROS production and recovering antioxidant levels. The improved seminiferous tubule structure and presence of spermatozoa support the enhanced sperm parameters, while the preservation of Leydig cells corresponds with increased testosterone levels (Hadwan et al., 2024).

Conclusion

This study aimed to investigate the modulatory effects of hydromethanolic leaf extract of *Costus afer* on fasting blood glucose, oxidative stress biomarkers, and reproductive parameters in streptozotocin-induced diabetic mice. The findings demonstrate that the extract provides significant protection against diabetes-induced metabolic and reproductive dysfunction by reducing fasting blood glucose levels, attenuating lipid peroxidation, and restoring antioxidant enzyme activities. Improvements in sperm count, motility, morphology, and serum testosterone levels further indicate preservation of testicular integrity and enhanced reproductive function. These effects are likely mediated through suppression of hyperglycemia-driven reactive oxygen species generation and strengthening of endogenous antioxidant defenses, with dose-dependent efficacy comparable to standard therapy at higher concentrations. Also, the histopathological results provide a structural basis for the biochemical and functional findings, confirming that the protective effects of *C. afer* are mediated through attenuation of oxidative stress and preservation of testicular cellular integrity. The dose-dependent histological recovery strengthens the observed improvements in antioxidant status, sperm quality, and hormonal balance, thereby validating the therapeutic potential of the extract in managing diabetes-induced reproductive toxicity. Based on these findings, further research is recommended to isolate and characterize the active phytochemical constituents, elucidate underlying molecular mechanisms, and assess long-term safety. In addition, well-designed clinical studies are needed to validate its therapeutic potential and support its development as an adjunct intervention for diabetes-associated male infertility.

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Conflict of interest

The authors confirm that there is no conflict of interest involve with any parties in this research study.

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