



***Lasianthera africana* leaves mitigate the toxicity of diabetes on the testicles in Wistar rats.**

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Abstract

Objective: In Nigeria, testicular toxicity is a known side effect of diabetes mellitus, one of the disorders for which the leaves of *Lasianthera africana* are used in ethnomedicine. This study evaluated the leaf extract and its fractions' ability to mitigate the toxicity of diabetes-induced testicular damage in rats.

Materials and Method: Wistar rats were given a high-fat diet and a modest dosage of streptozotocin to induce diabetes. Six groups of animals were used: Group 1, the drug-free group, received 10 mL/kg of DMSO; group 2, the diabetic control group, also received 10 mL/kg of DMSO; rats in group 3 received 10 mg/kg of glibenclamide as a positive control. Rats in groups 4, 5, and 6 were given crude extracts of *L. africana* leaves at a dose of 400 mg/kg, along with 30% and 100% methanol fractions.

Results: When comparing diabetic control rats to normal control, sperm indices were compromised, testicular sizes dropped, testicular architecture deformed, and sex hormone levels considerably ($p>0.05$) decreased. As glibenclamide was administered to diabetic rats, the above parameters showed a significant ($p<0.05$) improvement, as did the fractions and crude extract of *L. africana* leaves as compared to the diabetic control group. Rats treated with 100% fraction showed effects similar to those of glibenclamide. There were common bioactive metabolites in the plant leaves.

Conclusion: Results demonstrate that *Lasianthera africana* leaf fractions and crude extract reduced the harmful effects of diabetes mellitus on rat testicles, with 100% of the fraction having effects comparable to those of glibenclamide.

Keywords: *Lasianthera africana*; Diabetes; Testicular; Toxicology; Rats

Introduction

Diabetes mellitus, sometimes referred to as chronic metabolic disease, continues to be a serious global health issue. The incidence of this disease has steadily increased during the last few decades. Its prevalence has been linked to a number of factors, including obesity, sedentary behavior, and



poor lifestyles. It is estimated that by 2045, there would be 783.2 million people living with diabetes mellitus, up from 536.6 million in 2021(1).

This disease has a negative economic impact and is linked to higher rates of morbidity and mortality, particularly in poor nations. Patients with diabetes mellitus still face numerous obstacles despite the availability of numerous treatment options, such as unfavorable side effects, expensive costs, and restricted accessibility. The management of diabetes mellitus has seen a rise in interest in complementary and alternative medicines as a result of these issues, as well as unfulfilled expectations and insufficient efficacy(2).

Male infertility is a result of diabetes mellitus impairing male reproductive functions, as observed in both people and experimental animals (3, 4) There are three possible mechanisms for infertility: pre-testicular, and post-testicular (5). There are several known mechanisms that lead to testicular injury brought on by diabetes. Enhanced oxidative stress, weakened antioxidant defenses leading to increased ROS production and high lipoperoxidation in the seminal fluid, sperm DNA fragmentation due to gene expression defects in sperm DNA repair, respiratory chain alterations and mitochondrial DNA deletions, and end product enzymatic glycation are among them (6-8). These processes impact spermatogenesis, which results in low motility, count, and quality of sperm, among other indicators (9, 10). If diabetes mellitus is appropriately managed, complications caused by the condition can usually be avoided, reduced, or even completely reversed. The indigenous people has utilized several medicinal plants found in the tropical rainforest, such as *Lasianthera africana*, to treat diabetes mellitus.

The perennial glabrous herb *Lasianthera africana* (Icacinaceae) grows to a height of 3-6 meters. It is found in the thickets and secondary jungles of the rain forests of Southern Nigeria, Western Cameroon, and even Zaire. It is frequently seen in the Nigerian states of Akwa Ibom and Cross River. The leaves, fruits, and roots of the plant are among the many parts that have been utilized in traditional medicine. Its four ethnovarieties of leaves, which differ in taste, color, and ecological range, are consumed as vegetables. It has been stated that the natives use the fruits to cure wounds, skin conditions, hypertension, and asthma (11, 12). Numerous biological activities are exhibited by plant leaves, such as antibacterial, hypoglycemic, antiplasmodial, antioxidant, and antiulcerogenic properties (13-17). The plant's leaf is harmless, according to Inyang et al (18), with an LD50 of more than 5000 mg/kg body weight. There have been reports of phenolic component



richness in the plant (19, 20). Despite claims that plant leaves have antidiabetic properties, this study examines the plant's fractions and crude extract's capacity to mitigate the rat model of diabetes-induced testicular damage.

Materials and methods

Plant material collection and authentication:

In December 2017, leaves of *L. africana* were obtained from a local market in Calabar Municipality, Cross River State, and verified at the University of Calabar's Botany Department in Nigeria. The plant voucher specimen (EUDB S01/41) was kept in that department's herbarium.

Making the fractions and crude extract from the plant's leaves:

The leaves were allowed to air dry in the lab before being ground into a coarse powder in a grain mill that was run by hand (Corona®, Columbia). Each leaf powder sample weighed five hundred milligrams. A solvent was made by mixing 50 milliliters of methanol and 50 milliliters of dichloromethane in a 1:1 ratio. After letting the combinations remain for 48 hours, Whatman No. 1 filter paper was used to filter them. Crude extracts were created by concentrating the filtrates in a thermoregulated water bath (44 °C), and these concentrates were then stored in the refrigerator until they were needed for the task. Column chromatography was used to create the fractions, slightly altering Jin and Russell's ²⁰ procedure. A slurry consisting of 14 grams of silica gel powder (mesh size 120-200) was prepared at room temperature for 10 minutes using distilled water, and the resulting mixture was then poured into a cylindrical column measuring 50mm in diameter and 1cm in height. Ten milligrams of the above-mentioned paste crude methanol and dichloromethane extract were dissolved in 30 and 100% methanol, respectively, and added to the top of the corresponding columns. The plant's methanol was placed onto the packed column at 30% and 100%, respectively, and allowed to pass through the column. As the solvent elutes from the bottom of the column, the eluent is collected. The fractions were employed as 30% and 100% methanol fraction stocks, respectively, after being collected and concentrated in a thermoregulated water bath.



Phytochemical analysis:

Standard protocols and assays were used to perform a phytochemical analysis on the leaf extract of *L. Africana* (21).

Experimental animals:

For the investigation, male Wistar rats weighing between 170 and 190 g were recruited from the University of Calabar's Department of Pharmacology's animal house. The animals were kept in an air-conditioned room with good ventilation, where they were fed a regular pellet rat diet (Oladokun feed, Ibadan, Nigeria), and had access to unlimited water. The temperature was kept between 25 and 27 °C and the relative humidity between 45 and 55% for a duration of 12 hours. The animal protocol used in this investigation was adapted from the National Institute of Health Guide for the Care and Use of Laboratory Animals (22). Every attempt was taken to reduce animal suffering, and the study was carried out in compliance with the University of Calabar's Animal Ethical Committee recommendations (UNICAL/010/PA/2016).

Acute toxicity test:

Using OECD TG 423, the LD50 of *L. africana* leaf extract was measured to ascertain the agent's acute toxicity (23). The research was done in two stages, using 180–200 g male and female rats. The leaf extract was given orally to three sets of three rats (male and female separated in each cage) at doses of 100, 600, and 1000 mg/kg. For a full day, they were watched for indications of toxicity and death, with the first four hours receiving particular care. The extract (2000, 3000, and 5000 mg/kg) was then given to the next three groups of three rats, and as was previously said, equal observation was maintained. Salivation, paw-licking, writhing, change in body weight, and mortality are examples of signs of poisoning that were seen during 24 and 48 hours, respectively. The ultimate LD50 values were computed after recording the number of deaths in each group.

Induction of experimental diabetes:

Dina et al.'s (24) approach of high-fat diet (HFD) and low-dose streptozotocin (STZ) was used to induce diabetes. For 21 days, the rats were fed a high-fat diet that was prepared according to the previous instructions (25). A low dose of STZ (30 mg/kg body weight) diluted in 0.1M sodium citrate buffer (pH 4.7) was given intraperitoneally on the morning of the 22nd day. Rats' fasting



blood glucose levels were measured 48 hours after injection using a glucometer and blood from the ends of their tails. Animals used in the study were considered diabetics if their fasting blood glucose level was greater than 200 mg/dl (26). Animal treatments: Sixty-six rats—six diabetic and thirty non-diabetic—were divided into groups of one, two, three, four, five, and six. Thirty diabetic rats were split into groups 2 through 5, whereas the six non-diabetic rats made up group 1 (Normal control). To reconstitute the extract, dimethylsulphoxide (DMSO) was used. Rats in diabetic control groups 1 and 2 were given 0.5 milliliters of DMSO. Glibenclamide (10 mg/kg) was given to rats in group 3, and rats in groups 4, 5, and 6 received crude extract from *L. africana* leaves, 100% fraction, and 30% fraction, respectively. For 28 days, the 400 mg/kg dose of crude extract and fractions was administered by stomach canula. The rats were humanely euthanized on the 29th day following an overnight fast, and blood was extracted via heart puncture into plain bottles to prepare sera for hormonal analysis. The animals were given light chloroform anesthesia during this procedure. The testes and epididymis were removed through an abdominal incision made in the midline.

Hormonal assay

Using the appropriate enzyme-linked immunosorbent assay (ELISA) kits, sera from various experimental groups were utilized to assess the levels of FSH, LH, and testosterone (Life Science Inc., USA). Every test was conducted in compliance with the manufacturer's instructions.

Evaluation of Sperm Characteristics

Using an automated sperm analyzer, semen was extracted from the epididymis in accordance with a previous description by Essiet et al. (27). The sperm's morphological features were assessed using a light microscope.

Assessment of rat testes

Rat testes were extracted, cleaned in between filter papers, and cleared of any extraneous tissues before being assessed. A pair of vennier callipers was used to measure the diameters and lengths, and a sensitive electronic weighing scale was used to measure the weights. Every rat was measured



in both testes, and the average values for every parameter were considered as a single observation. The mean value of every parameter for every group was computed based on these observations. After that, the testes were imbedded in paraffin and fixed in newly made 10% formalin. Hematoxylin and Eosin (H&E) was used to prepare tissue slices with a thickness of 5 microns. The tissue sections were then seen under a light microscope (Olympus/3H, Japan) with a magnification of 400 and photomicrographs were taken. Four parts, one from each of the testis's upper, middle, and lower poles, were obtained from various fields to provide a representative representation of each testes.

.Analytical methods

One-way analysis of variance (ANOVA) and Turkey's multiple comparison post hoc test were used in the statistical software for social sciences (SPSS version 20) analysis of the means \pm SEM data. A $P < 0.05$ indicated a statistically significant difference.

Results

Phytochemical composition: The crude leaf extract and fractions of the plant contained common secondary plant metabolites (Table 1).

Acute toxicity test: Even at a dosage of 5000 mg/kg body weight of the crude leaf extract and fractions of *L. africana*, there were no outward manifestations of toxicity. In rats, the LD50 was deemed to be higher than 5000 mg/kg.

Effect of various treatments on rat testicular parameters: Testicular parameters (weight, diameter, and length) significantly decreased ($p < 0.05$) in diabetic control rats when compared to normal control rats, but these parameters increased ($p < 0.05$) in rats treated with glibenclamide and *L. africana* leaves when compared to diabetic control rats (Table 2).

Rat sex hormones in response to various treatments: The findings are shown in Table 3. Rats with diabetes control had significantly ($p > 0.05$) lower serum levels of testosterone, FSH, and LH than normal control rats. The three hormone levels were considerably ($p < 0.05$) higher in rats



treated with glibenclamide and *L. africana* leaves than in DC. The 100% fraction value was most similar to glibenclamide.

Impact of various treatments on the sperm characteristics of rats: Tables 4 and 5 provide an overview of this. When comparing diabetic control rats to normal control, there were substantial ($p < 0.05$) declines in the total epididymal sperm count, sperm motility (%), and sperm viability (%). When glibenclamide (group 3), crude extract, and fractions of *L. africana* leaves were administered to diabetic rats, their parameters considerably increased in comparison to the diabetic control group (Table 4). The same trend was seen in the effects of treatments on sperm morphology (Table 5).

Table 1. crude extract and fractions of leaves of *L. africana* that include phytoconstituents.

Constituent	Crude extract	30% fraction	100% fraction
Alkaloids	++	++	++
Anthraquinone	++	+	+
Saponnins	++	+	+++
Flavonoids	++	++	+++
Glycosides	+	+	+
Tannins	+	++	++
Phenol	++	++	+++

Very abundant = +++; Moderately abundant = ++; Detected = +



Table 2. Effects of various therapies on testicular parameters in rats

Treatment	Weight of testes (g)	Length of testes (mm)	Diameter of (mm)
Normal control 10 mL/kg	1.55±0.16	21.85±1.02	12.77±0.25
Diabetic control 10 mL/kg	0.78±0.33	15.12±0.04	7.61±0.02
Glibenclamide 10 mg/kg	1.39±0.19 ^a	18.73±0.20 ^a	10.84±0.41 ^a
30% LAL fraction 400 mg/kg	1.12±0.29 ^a	17.67±0.23 ^a	10.17±0.49 ^a
100% LAL fraction 400 mg/kg	1.22±0.13 ^a	18.88±0.18 ^a	10.84±0.16 ^a
LAL Crude extract 400 mg/kg	1.05±0.25 ^a	18.02±0.20 ^a	10.33±0.67 ^a

Values are expressed as mean ±SEM (n=6) ^a p<0.05 vs Diabetic control;

LAL = *Lasianthera africana* leaf

Table 3. Different therapies' effects on male rat sex hormones

Treatment	Testosterone (ng/ml)	FSH (ng/ml)	LH (ng/ml)
Normal control 10 mL/kg	0.28±0.07	3.88±0.45	6.47±0.53
Diabetic control 10 mL/kg	0.22±0.01	2.18±0.11	4.25±0.49
Glibenclamide 10 mg/kg	0.28±0.15	3.63±0.20 ^a	5.74±0.49
30% LAL fraction 400 mg/kg	0.27±0.21	3.67±0.13 ^a	5.70±0.49
100% LAL fraction 400 mg/kg	0.29±0.13	4.10±0.18 ^a	6.40±0.16 ^a
LAL Crude extract 400 mg/kg	0.27±0.25	3.82±0.20 ^a	5.73±0.67 ^a

Values are expressed as mean ±SEM (n=6) ^a p<0.05 vs diabetic control



LAL = *Lasianthera africana* leaf

FSH = Follicle stimulating hormone; LH = Luteinizing hormone

Table 4. Different treatments' effects on the properties of rat sperm

Treatment	Count (10 ⁶)	Motility (%)	Viability (%)
Normal control 10 mL/kg	23.30±0.18	77.55±1.02	82.61±1.25
Diabetic control 10 mL/kg	10.01±0.22	31.92±2.03	35.49±2.11
Glibenclamide 10 mg/kg	18.58±0.16 ^a	41.32±5.02 ^a	58.77±2.20 ^a
30% LAL fraction 400 mg/kg	17.95±0.33 ^a	39.53±2.14 ^a	53.88±0.36 ^a
100% LAL fraction 400 mg/kg	20.03±0.42 ^a	42.11±1.89 ^a	57.01±0.22 ^a
LAL Crude extract 400 mg/kg	16.81±0.19 ^a	35.11±3.02 ^a	50.96±0.11 ^a

Values are expressed as mean ±SEM (n=6) ^a p<0.05 vs diabetic control

LAL = *Lasianthera africana* leaf

Table 5. Different treatments' effects on the morphology of rat sperm

Treatment	Normal (%)	Abn head (%)	Abn body (%)	Abn tail (%)
Normal control 10 mL/kg	79.42±1.25	7.25±1.25	4.08±0.63	9.25±1.25
Diabetic control 10 mL/kg	35.71±0.95	24.46±1.05	12.48±1.33	27.35±1.09
Glibenclamide 10 mg/kg	64.54±0.66 ^a	10.36±1.05 ^a	9.95±1.21 ^a	15.15±1.15 ^a
30% LAL fraction 400 mg/kg	59.24±0.62 ^a	10.26±1.05 ^a	12.15±1.28 ^a	18.35±1.55 ^a
100% LAL fraction 400 mg/kg	63.54±0.62 ^a	10.10±1.04 ^a	10.21±1.00 ^a	16.15±1.25 ^a
LAL crude extract 400 mg/kg	56.58±0.39 ^a	13.96±1.19 ^a	12.47±1.66 ^a	16.99±1.45 ^a



Values are means \pm SEM. (n=6) ^a = p<0.05 vs Diabetic control

LAL = *Lasianthera africana* leaf

Abn = abnormal

Effects of various treatments on the rat testicular histology: Results are shown in Figs. 1 through 6. The normal control rat testes (Fig. 1) had a section showing intact basement membrane-containing, healthy seminiferous tubules that contained spermatogonial cells at different stages of maturation (spermatogonial A and B, primary and secondary spermatocytes, spermatids, and spermatozoa). The leydig and interstitial cells were in a normal state. The seminiferous tubules in the diabetes control group (Fig. 2) had few spermatogonial cells (mostly spermatogonial A and B) and poor interstitial space between cells, densely packed leydig cells, and a thicker basement membrane. Rats treated with glibenclamide possessed seminiferous tubules (Fig. 3) with a unique basement membrane, layers of spermatogonial cells at different stages of maturity, few leydig cells, blood vessels, and spermatozoa that were sparsely populated. The testes of diabetic rats administered with *L. Africana* leaf fractions and crude extract (Figs. 4, 5, 6) showed histological characteristics resembling those of rats treated with glibenclamide.

Fig 1

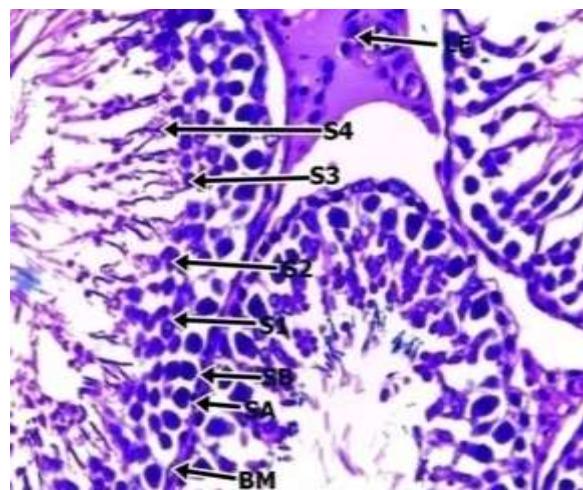


Fig 2

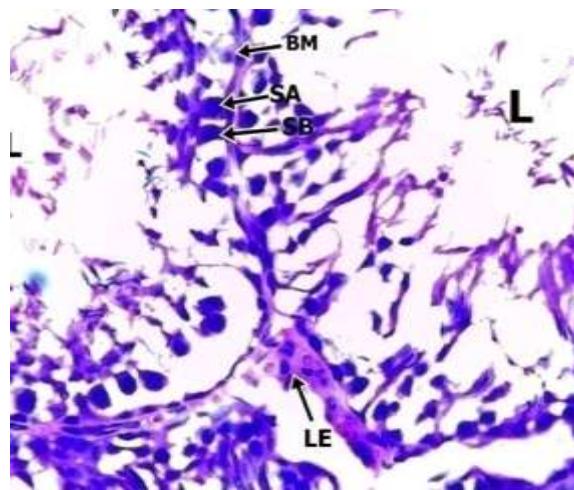




Fig 3

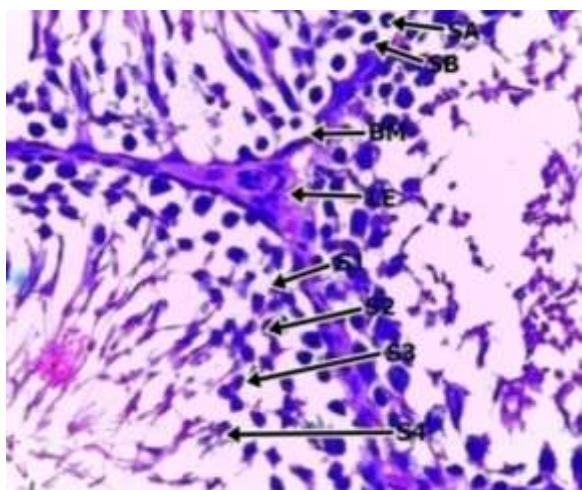


Fig. 4

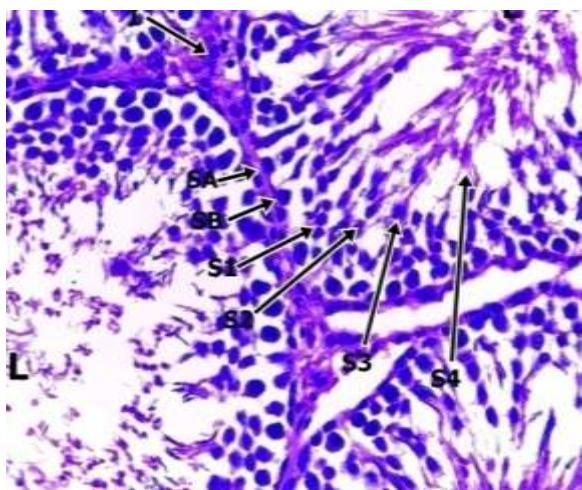


Fig. 5

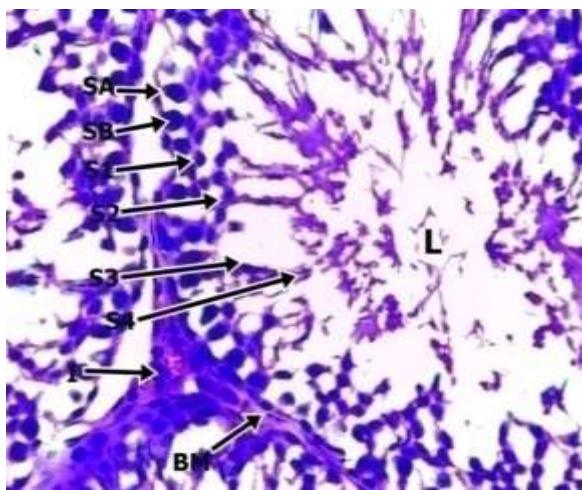
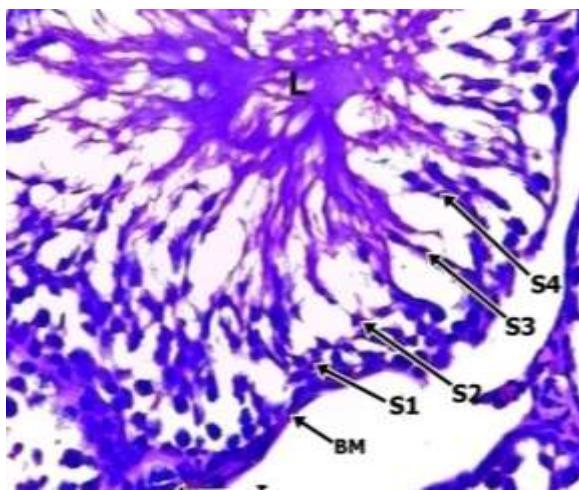


Fig. 6





Photomicrographs of slices of testicular tissue from rats (H&E stain x 400): The healthy seminiferous tubules with intact basement membranes including various phases of spermatogonial cells were depicted in Fig. 1 (Normal Control). Rats in Fig. 2 (Diabetic control) showed disturbed seminiferous tubules with sparsely populated leydig cells, poor intervening interstitium, and few spermatogonial cells. A distinct basement membrane, layers of spermatogonial cells at different stages of development, few leydig cells, blood vessels, and sparsely populated spermatozoa were all present in the seminiferous tubules in Fig. 3 (5 mg/kg glibenclamide-treated group). Figure 4 (the diabetic plus 30% LA component) seminiferous tubules with spermatogonial cells at different stages of development (spermatogonial A and B, primary spermatocytes, secondary spermatocytes, spermatid and spermatozoa), lumen containing spermatozoa, intervening interstitium with scanty blood vessels, and leydig cells were all shared by Figs. 5 (diabetic + 100% of LA leaf) and Fig 6 (Diabetic + LA leaf).

Legend: S1 stands for primary spermatocyte, S2 for secondary spermatocyte, SB for spermatogonial B, SA for spermatogonial A, S4: mature spermatozoa, S3: spermatids, LE is the Leydig cell; BM is for basement membrane; ST stands for seminiferous tubules.

Discussion

The search for an agent capable of reversing the damage is becoming increasingly urgent as testicular dysfunction caused by diabetes mellitus increases. This study has demonstrated that fractions and leaf crude extract of *Lasianthera africana* ameliorate the testicular damage caused by diabetes mellitus in rats. The leaf has been reported to possess strong antioxidant activity, and to be rich in compounds with high antioxidant actions, such as phenols, flavonoids, and saponins (28). These phytochemicals scavenge ROS which cause oxidative stress, a major predisposing factor to DM-induced testicular dysfunction (6).

Our test plant leaves underwent chemical screening, which produced bioactive agents such as the previously mentioned compounds. These compounds were abundant, particularly in rats treated with 100% fraction, and they could be the reason behind the observed improvement in testicular parameters and functions in diabetic rats treated with various plant leaf preparations as compared to the diabetic control group. Additionally, diabetic rats treated with glibenclamide demonstrated signs of improved testicular functions.



When low dose STZ and HFD are combined to induce experimental diabetes, this drug may correct the defective insulin secretion caused by STZ and depressed insulin resistance to peripheral tissues by improving the sensitivity of existing insulin receptors and increasing insulin secretion from pancreatic β -cells (29).

The amount of secretory and metabolic cells in the testis is reflected in its size, making it a sensitive instrument for evaluating its function (30). Testicular dysfunction, a known symptom of diabetes mellitus, as indicated by the study's significant ($p<0.05$) reductions in testicular sizes (weight, length, and diameter) of diabetic control rats compared to normal control rats. This shows that low dose streptozocin and HFD were successful in inducing DM and the testicular damage that goes along with it in this study. A reversal of the caused testicular damage is suggested by the rise in testicular parameters shown in diabetic rats treated with glibenclamide, fractions, and crude extract

of *Lasianthera africana* leaves. Studies employing curcumin and sesame oil have produced results that are comparable (31, 32).

Sperm analysis has a significant impact on fertility because it provides an overview of spermatogenesis at all stages as well as the motility, viability, and morphology of the produced sperm cells (33). Previous studies have shown that diabetes mellitus impairs spermatogenesis, resulting in poor sperm indices (6, 31). This was the case in our study, where the sperm indices (count, viability, motility) of the diabetic control rats were significantly ($p<0.05$) lower than those of the normal control group. These indices were raised in the diabetic rats treated with various preparations of *Lasianthera africana* leaves, and the results were comparable to those of the glibenclamide-treated group.

One possible explanation for the enhanced sperm indices is that components of the plant leaf extract were able to scavenge reactive oxygen species (ROS) in the seminal fluid, thereby halting more damage to the seminiferous tubules. Additionally, since the inhibition of these systems permits the accumulation of ROS leading to oxidative stress, it is plausible that the leaf extract improved antioxidant mechanisms in rat seminal fluid (34).

Similar to earlier studies' findings,³⁵ the study's diabetic control rats had lower serum levels of testosterone, FSH, and LH than the normal control group. Rats treated with glibenclamide and those treated with extracts or fractions had significantly higher levels of these hormones ($p>0.05$)



when compared to the diabetes control group. Given that diabetes is known to impair the hypothalamo-hypophyseal-testicular axis' functions, including the release of GnRH (35), the observed increase in hormone levels in the rats treated with extract or fractions may indicate that this axis is stimulated, which would reverse the depression that is caused by diabetes mellitus. Gonadotropes in the adenohypophysis produce luteinizing hormone (LH) and follicle stimulating hormone (FSH) in response to GnRH. When compared to normal control rats, the diabetic control rats' lower serum levels could indicate low secretion due to either a low density of gonadotrophs

or a reduced release of GnRH. By increasing the density of gonadotrophs or improving GnRH release, the leaf preparations may have caused hormonal increases based on the information provided above. Given that FSH is essential for sperm development, the low levels in diabetic control rats may help to further explain the observed impairment in sperm indices (36). The elevated levels observed in glibenclamide-treated diabetic rats and the leaf preparations also account for the improvement in sperm parameters.

Testicular dysfunction is indicated by morphological indicators such as atrophy of seminiferous tubules and loss of spermatogenetic cells. Research by Cameron et al. (37), has demonstrated that diabetes is a cause of both of these symptoms. In this study, the distorted shape of seminiferous tubules and a significant decrease in the number of spermatogenic cells in the diabetic control rats were compared to normal rats and indicated disordered testicular morphology. This is consistent with Ghafari et al.'s findings (4). Glibenclamide, in combination with the crude extract and plant leaf fractions, reversed the damage to the testes.

Conclusion

The results of this study demonstrates that the *L. africana* leaf fractions and crude extract improve sperm parameters, testosterone, LH, and FSH levels, and testicular indices (weight, length, and diameter)—all of which are negatively impacted by diabetes on rat testes. Histological analysis suggests that *L. africana* leaves are favorable for spermiogenesis in diabetic rats, but more microscopic analysis is required for a solid confirmation. A promising therapeutic agent for the prevention or treatment of diabetes-induced testicular injury is the plant leaf.



Declarations

Acknowledgement

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Compliance with ethical guidelines

The study was conducted according to the Animal Ethical Committee guidelines of University of Calabar (UNICAL/010/PA/2016), and every effort was made to minimize animal suffering.

Conflict of interest statement

We declare that there is no conflict of interests.

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Authors' contributions

Study design: Essiet GA and Eko WO; The literature search and editing of the manuscript:

Akuodor GC, Ofor CC and Ohanme EO; Statistical analyses: Eze CE, **Imakwu II, Asika EC** and Nwadum SK; Experiments and final approval: All authors;

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