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AMELIORATIVE EFFECTS OF ROOT BARK EXTRACTS OF *FERETIA APODANTHERA* ON REPRODUCTIVE PARAMETERS IN STREPTOZOTOCIN INDUCED DIABETIC MALE WISTAR ALBINO RATS

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Abstract
Diabetes mellitus is a chronic illness that is relatively prevalent. Employing routine procedures, the reproductive parameters of Feretia apodanthera root bark extracts in male wistar albino rats induced with streptozotocin (STZ) were examined. The root bark of the plant was air-dried and subjected to Soxhlet extraction. The invitro antioxidant potential of the extract was investigated using DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity. While lipid profiles, sperm parameters and histopathology were conducted using standard procedures. Diabetes induction of male wistar albino rats was carried out with 50 mg intravenous streptozotocin injection. Seven groups were randomly assigned to the rats (A, B, C, D, E, F and G).Group A (normal control) received 1 ml of distilled water (vehicle), B (Diabetic induced untreated), groups C and D were supplemented with 100 and 200mg/kg body weight (b.w) aqueous extract, while group E and F were administered 100 and 200mg/kg body weight ethanol extract, and group G received a combined dosage of 5mg/kg b.w sildenafil citrate + 100mg Metformin. The percentage yield obtained following extraction was found to be 5.91%, 5.52% and 2.14% (w/w) for n-hexane, aqueous and ethanol root bark extracts of Feretia apodanthera respectively. DPPH radical scavenging activity reveals that aqueous extract has a higher antioxidant potential than ethanol and n-hexane extract. Diabetic rats treated with aqueous and ethanol extracts shows significant ($p<0.05$) improvement of body weight compared to the untreated groups. Total cholesterol, triglycerides and low-density lipoprotein cholesterol were significantly ($p<0.05$) lower in aqueous extracts (200mg/kg b.w) while high density lipoprotein cholesterol was significantly ($p<0.05$) higher compared to the diabetic control group. Likewise, diabetic rats treated with 200mg/kg b.w of aqueous shows significant ($p<0.05$) and preferable healthy risk at herogenic predictors, while groups treated with ethanol extract shows no significant ($p<0.05$) difference in all the predictor indices compared with STZ induced untreated group. The level of testosterone and sperm parameters were significantly($p<0.05$) improved in aqueous extract treated group while histopathology of the testes reveals a better appearance of the spermatogenic cells and seminiferous membranes in aqueous extract treated group which is synonymous to the normal control. The result implies that root bark aqueous extract of Feretia apodanthera, particularly-200mg/kg b.w had significant potential in ameliorating sexual impairment in diabetic male rats.
Key words: *Antioxidants, sperm, roots, lipids, & testosterone.*

Introduction

Diabetes Mellitus is a metabolic disorder caused by absolute or relative deficiency of insulin and insulin resistance. Diabetes affects about over 529 million people worldwide, including men, women, and children of all ages. It is anticipated that the number will surpass double, to 1.3 billion by 2050 (GBD, 2021). With a 6.1% worldwide incidence rate, diabetes is currently within the top ten (10) main causes of death and disability (IDF, 2022). Cardiovascular diseases, myocardial infarction, neuropathy, nephropathy, retinopathy & reproductive dysfunction are few amongst the complications of diabetes. Evidence has shown that there is a strong correlation between diabetes and male sexual dysfunction (Deffeudis *et al.*, 2021).Diabetes mellitus has a negative impact on the male reproductive system, causing testicular germ cells to degenerate, suppression of spermatogenesis, decrease in libido, levels of testosterone, and seminiferous tubule diameter (Kermani *et al.*, 2019).Hyperglycemia stimulation up-regulates the number of mitochondria in tissue cells. Reactive oxygen species (ROS) are the primary contributor of damage to cells. Lipid peroxidation damages the

antioxidant defense system (Su *et al.*, 2019). It was further revealed that men with diabetes had an increased risk of sexual dysfunction and that it manifested sooner in life than men without diabetes (Maiorino *et al.*, 2014).It has been shown that streptozotocin (STZ) induction in rats and different animals is a successful model for diabetes. Nevertheless, excessive dosages of STZ may cause harm to tissues other than the pancreas. This include seminiferous tubules, body, and weight of sexual organs, circulating levels of testosterone, and parameters associated with sperm were all dramatically changed by STZ-induced diabetes (Imaeda *et al.*, 2002).

The bushy, deciduous shrub *Feretia apodanthera* belongs to the *Rubiaceae* family and has branches that sway or twist, and it can grow up to 6 metres tall. The wild plant is gathered and used locally for medicinal purposes, food, and cosmetic products (Le Houre, 1978). They occur naturally in the vicinity of clay soils and soils that frequently flood during the seasons. The crimson, luscious pulp of ripe fruit is eaten raw as a snack and utilised as a thirst quencher, especially by children and shepherds. Because they are commonly available, affordable, and less harmful, the preliminary examination of this plant and its pharmacological component for sex-enhancing impacts in diabetics might boost curiosity regarding usage for managing male sexual dysfunctions. The plant's root bark extracts are used in traditional medicine to supplement male sexual power. Limited research has been reported on the plant's spermatogenic effects in a diabetic model, hence this study is being conducted in male wistar rats that have been induced with streptozotocin (STZ), to examine any potent beneficial effects of *Feretia apodanthera* extract against sexual impairment.

Materials and Methods

Animals

The Canadian Council of Animal Care and the United States National Institute of Health have issued International, National, and Institutional Guidelines for the care and use of Laboratory Animals in Biomedical Research, which were followed during the experimental procedures involving the animals and their care (NRCC, 2011). A total of 108 Wister albino rats, weighing between 150 and 180 grammes on average and belonging to both sexes, were acquired and housed in an animal housing with adequate ventilation within the Department of Pharmacology and Pharmaceutical Sciences at Ahmadu Bello University in Zaria, Nigeria. Before the trial started, the animals were given two weeks to adapt to their new surroundings. Every day, they were given water and grower mash from Nigeria's Vital Feeds Company.

Drugs and Supplies.

Progesterone, estradiol benzoate, streptozotocin (STZ), ethanol and n-hexane were all supplied by Sigma Chemical Company, St. Louis, U.S.A., while a Standard Pharmaceutical store supplied the metformin and sildenafil citrate. All chemical compounds/reagents utilized were analytically graded.

Sample Collection and Preparation.

Fresh *Feretia apodanthera* roots were collected from Magami village, Gusau local government area, Zamfara State, Nigeria, which is the species' natural habitat. The plant was recognizedand verified by a taxonomist at Ahmadu Bello University's Herbarium Unit in the Biological Sciences Department in Zaria, Nigeria, where a voucher specimen bearing the number 930 was placed. Using an iron tool, the bark was removed from the roots, and it was allowed to air dry for three weeks at room temperature. Dry samples were crushed into a powder using a mortar and pestle after three weeks. After the powder was sieved through a 1-mm mesh sieve, dried root bark powder (500g) of *Feretia apodanthera* was extracted successively with 2500ml each of n-hexane, ethanol, and aqueous solution in a soxhlet

extractor. The solvent was removed by distillation and the semisolid mass was dried using a hot water bath. The three extracts obtained without the solvent were weighed and the percentage yields were calculated in terms of air-dried weight of the plant material. All the extracts were dissolved in 1% normal saline before the commencement of treatment.

In vitro Antioxidant Capacity

The DPPH radical scavenging activity of the root's bark extracts of *Feretia apodanthera* was assayed by the DPPH radical scavenging activity assay (Chan *et al.*2007).

DPPH solution was prepared by dissolving 6 mg of DPPH in 100 ml of methanol. To 1ml of various concentrations of the extracts (20, 40, 60, 80, 100 µg/ml), 2 ml of DPPH solution (0.1mM) was added. An equal amount of methanol and DPPH served as control. The mixture was shaken vigorously and was left to stand in dark for 30min. The absorbance of the resulting solution was measured spectrophotometrically at 520 nm. The experiments were performed in triplicate and the percentage scavenging activity of each extract on DPPH radical was calculated using the following formula.

$$I = \frac{\{(Ab - Ae)\}}{Ab} \text{X100}$$

Where I, is the percentage of inhibition, A_b absorbance of the blank sample and A_e is the absorbance of the extract.

Acute toxicity test

According to Organization for Economic Cooperation and Development (OECD) recommendations guideline, (AOT 425), specifies that an acute toxicity study was conducted on adult male albino rats in good health. The rats were observed for two hours nonstop for autonomic and behavioral patterns, and for any additional signs of toxicity or mortality for a maximum of seven days (OECD, 2001).

Induction of Diabetes

For the experiment, male rats weighing between 150 and 180 grammes were chosen and subjected to overnight fasting. They were always given water during the 18-hour fast. Streptozotocin (50 mg/kg, IV) in citrate buffer (pH 4.4, 0.1M) was given to the rats. Blood samples were taken, and their blood glucose levels were examined three days later. In this investigation, rats with blood glucose levels higher than 200 mg/dl were included (Ghasemi *et al.*, 2023). Twenty-four hours following the onset of diabetes, animals began receiving treatment with extracts and a combination of metformin and sildenafil citrate (100 mg and 5 mg/kg body weight/day), which was administered every day for 21 days.

Animal Groupings

Forty-two male rats were randomly grouped into seven (1–7) consisting of 6 animals each and appropriate doses of the extracts were administered orally per day for twenty-one days. Group I- Normal control rats given 5 ml (1% Normal saline): Group II- Diabetic rats treated with 5 mL (1% Normal saline). Group III and Group IV are diabetic rats treated with 100 and 200mg/kg body weight of aqueous extract of *Feretia apodanthera*: Group V and Group VII are diabetic rats treated with 100 and 200mg/kg body weight of ethanol extract of *Feretia apodanthera*: while Group VII are diabetic rats treated with standard drugs mixture (Sildenafil citrate-5mg and Metformin-100mg).

Biochemical Evaluation

On the final day of treatment, the rats were fasted all night. Blood was sampled via cardiac puncture while under a light general anaesthesia. After centrifuging the samples for 20 minutes at 2200 rpm to separate the serum, they were stored at -20°c for various biochemical analyses. Under strict adherence to the manufacturer's guide, a commercial ELISA Kit (Sigma-Aldrich) was utilised to measure testosterone, and lipid profile.

Semen Analysis

Sperm Count.

Following the last treatment, the cauda epididymis was cut into tiny pieces and placed in 1 millilitre of 1% normal saline. This was done to enable the spermatozoa to move through the medium, which contained 10% formalin in PBS (formaldehyde fixative). After diluting 100 µl of the liquid with 100 µl of distilled water, one drop of the sample was added on a Neubauer haemocytometer, and it was left to stand for seven minutes. A count was conducted on the sperm scattered in the chambers using an Olympus microscope (Tokyo, Japan). The settling sperm were then assessed and counted per 250 tiny squares using a haemocytometer (Haredy *et al.*, 2017).

Sperm Motility

To measure sperm motility, one drop of fresh semen and two drops of warm 1% normal saline were added on a slide that had been pre-warmed to 37 °C. At least 200 arbitrarily selected spermatozoa were assessed using an optical magnification (x400), according to the World Health Organization's manual standards. Sperms were classified as either progressive (swimming rapidly down a line); non-progressive (swimming slowly along a line, an arc, or an irregular line): Immotile- having minimal movement /immobile (Keyhanmanesh *et al.*, 2018).

Sperm Viability

Using eosin Y staining (5% in saline), viability was evaluated. 10 microliters of eosin were added to 40 microliters samples of the fresh sperm suspension and were placed on a glass slide and examined under a light microscope. After staining, live sperm remained unstained, but dead sperm were identified by any pink or red colouration. Each sample had at least 200 sperm counted in 10 randomly selected fields of vision, and the proportion of viable sperm was registered (Öztaş *et al.*, 2019).

Histopathology of the Testes

Upon completing the experiments, the animals from various treatment groups were put to death under mild anaesthesia, and the harvested testes were rinsed in 1% normal saline. In summary, the testes were preserved in 10% v/v buffered formaldehyde, dehydrated using increasing ethanol concentrations (70, 90, and 95% v/v), cleansed in xylene, and then embedded in paraffin wax (which has a melting temperature of 56 ^{0}c) with segments were slit at 5 µm using a rotatory microtone. To avoid the segments from separating from the slides during the staining process, they were floated out onto clean microscope slides that had been albumenized beforehand. They were left to dry at 37°c for two hours. Following staining, the slides were dehydrated by passing them through progressively higher concentrations of alcohol (20–100%), and they were then cleaned with xylene. The tissue segment was covered with basalm, a permanent mounting medium. The covering-mounting media was covered with a thin glass-coated slip, and the tissue segments underneath were left to dry. Later, it was observed on a research microscope and photomicrographs were taken in bright field at x200 (Khaneshi *et al.*, 2013).

Data Analysis

The data were analysed using GraphPad prism (10.0.1). The results were expressed as mean ± standard deviation (SD). Analysis of variance (ANOVA) was conducted. The differences between the various animal groups were compared using the Duncan multiple range test. p-value less than 0.05 was considered as significant ($p \leq 0.05$) (Mahajan, 1997).

Results.

Extraction and Radical Scavenging Activity.

The percentage yield obtained following extraction were found to be 5.91%, 5.52% and 2.14% (w/w) for hexane, aqueous and ethanol root bark extracts of *Feretia apodanthera* respectively. The percentage yield (w/w) of the extracts have n-hexane residue as the highest yield (5.91%), followed by aqueous extract (5.52%), and ethanol extract has the lowest yield (2.14%).To determine the half maximal inhibitory concentration (IC_{50}) of DPPH on the extracts, a plot of percentage inhibition against the concentration of extracts was used to determine the antioxidant potential as shown in Figure 1.0. The free radical scavenging activity of aqueous, ethanol, n-hexane extracts reveal that aqueous extracts possessed the highest antioxidant activity at a concentration of 0.1 mg/ml. The IC_{50} value of all the extracts and Vitamin C were in the following order- Vitamin C> Aqueous extract > Ethanol Extract > n-hexane extract as depicted in Table 1.0.

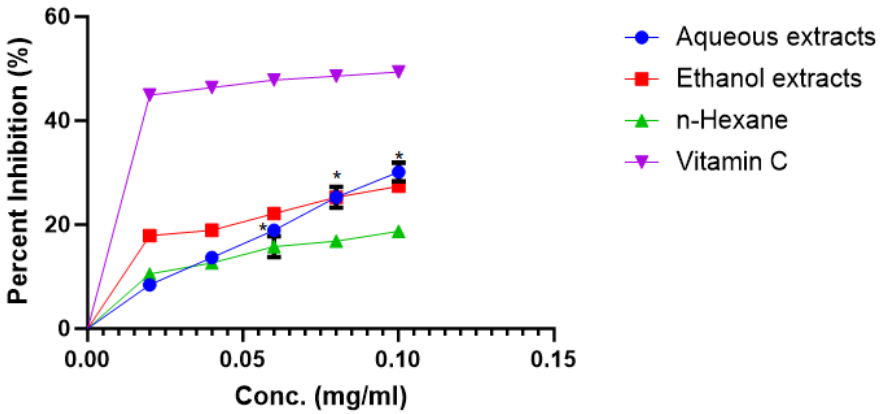


Figure 1.0: Free Radical Scavenging Capacity of various extracts of root bark of Feretia apodanthera using DPPH.

Table 1.0: IC_{50} Values of root bark extracts of *Feretia apodanthera* Compared to Vitamin C.

Extract	IC_{50}	Equation
Ascorbic Acid	0.0765	y=57.97x + 43.47
Aqueous Extract	0.1804	y = 257.89x + 3.4737
Ethanol Extract	0.2791	y = 126.32x + 14.737
n-Hexane Extract	0.6283	y = 63.158x + 10.316

Change in Body Weight.

Table 2.0 shows that the normal control group's relative body weight was significantly ($p<0.05$) higher than that of the negative control group. The diabetic control group of rats showed a significant decrease in weight ($p<0.05$). However, the diabetic rats supplemented with *Feretia apodanthera* root bark extracts (AE1, AE2, EE1, and EE2) showed a significant increase in body weight over the course of the experiment compared to the negative control group, with AE2 exhibiting the greatest improvement in body weight. Rats given the conventional medication (Sildenafil Citrate and Metformin) showed a noteworthy ($p<0.05$) increase in body weight in comparison to the negative control group.

Table 2.0: Effect of root bark extracts of *Feretia apodanthera* on body weights of STZ induced Diabetic Male rats.

GROUP	NC	DC	STZ+AE1	STZ+AE2	STZ +EE1	STZ+EE2	STZ+ STD DRGS
Initial Weight (g)	149.83±7.00	146.67±5.99	147.50±5.61	140.50±3.73	141.83±4.36	136.67±3.14	142.0±3.80
Final Weight (g)	170.67±6.95	127.33±4.72	153.00±3.35	155.33±5.28	145.50±4.14	143.67±2.88	157.00±6.13
Change in weight (%)	↑ 12.3	↓ 13.2	↑ 3.9	↑ 9.6	↑ 2.7	↑ 5.1	↑ 9.5

Values are represented as means +SD (n=6). NC- Normal control, DC-Diabetic control, STZ-Streptozotocin AE1-Aqueous extract(100mg), Aqueous extract (200mg), EE1-Ethanolextract (100mg), EE2-Ethanol extract(200mg), STD DRGS- Sildenafil citrate(5mg) + Metformin (100mg).

Lipid Profiles and Atherogenic Risk Predictors.

Diabetic rats treated with extracts and the standard drug combination treatment significantly ($p< 0.05$) lowered the triglycerides (TG), total cholesterol (TC), and low-density lipoprotein cholesterol (LDL-c) compared to the STZ induced untreated group. Only group administered aqueous extract (200mg/kg b.w) had significantly ($p< 0.05$) higher HDL-c compared to control and other treated groups as presented in Table 3.0. Furthermore, the serum atherogenic risk predictors were evaluated and showed that diabetic controls had significantly reduced level of HDL-c/TC, and significantly($p<0.05$) increased the level of LDL-c/HDL-c and TAG/HDL-c when compared to the normal rats group. Animal groups treated with 200mg/kg b.w aqueous extracts shows significant($p<0.05$) and preferable healthy risk predictors, while groups treated with ethanol extract shows no significant($p<0.05$) difference in all the predictor indices compared with STZ induced untreated group (Table 4.0).

Table 3.0: Lipid Profiles of Diabetic rats Fed with Various Concentrations of root bark extracts of *Feretia apodanthera.*

TREATMENT GROUP	TC(mmol/dl)	TG (mmol/dl)	HDL (mmol/dl)	LDL (mmol/dl)
NC	2.02 ± 0.08^{a}	0.50 ± 0.90^{a}	1.59 ± 0.12^{d}	0.92 ± 0.15^{a}
DC	2.85 ± 0.10^{e}	1.26 ± 0.21^{e}	0.85 ± 0.18^{a}	2.12 ± 0.26^{f}
STZ+AE1	2.60 ± 0.09^{c}	0.92 ± 0.12^{c}	0.85 ± 0.10^{b}	1.63 ± 0.21^{cd}
STZ+A.E2	2.28 ± 0.15^{b}	0.73 ± 0.14^{b}	1.13 ± 0.08^{c}	1.42 ± 0.15^{c}
STZ+E.E1	2.68 ± 0.12^{cd}	1.08 ± 0.07^{d}	0.61 ± 0.12^{a}	1.85 ± 0.19^{de}
STZ+E.E2	2.58 ± 0.15^{c}	0.90 ± 0.09^{c}	0.78 ± 0.07^{ab}	1.90 ± 0.18^{ef}
STZ+STD DRGS	2.33 ± 0.33^{b}	0.65 ± 0.10^{b}	1.18 ± 0.23^{c}	1.18 ± 0.15^{b}

Values are represented as means +SD (n=6). Values carrying different superscript letters along the column shows significant difference ($p<0.05$).NC- Normal control, DC-Diabetic control, STZ-Streptozotocin, AE1-Aqueous extract(100mg),Aqueous extract(200mg), EE1-Ethanol extract(100mg), EE2-Ethanol extract(200mg), STD DRGS- Sildenafil citrate(5mg) + Metformin (100mg).TC-Total cholesterol: TG- Triglyceride: HDL-High density lipoprotein cholesterol: LDL-Low density lipoprotein cholesterol.

Table 4.0: Effect of extract of root bark of *Feretia apodanthera* on Values of Serum Atherogenic risk Predictor Indices in Streptozotocin induced Diabetic Male rats.

TREATMENT GROUP	HDL-c/TC	LDL-c/HDL-c	TAG/HDL-c
NC	$0.73+0.07^{d}$	$0.63+0.11^{a}$	$0.34+0.61^{a}$
DC	$0.23+0.07^{a}$	$3.45+0.88^{d}$	$2.11+0.75^{c}$
STZ+AE1	$0.33+0.04^{b}$	$1.96+0.45^{c}$	$1.09+0.19^{b}$
STZ+A.E2	$0.50+0.05^{c}$	$1.25+0.11^{b}$	$0.64+0.09^{a}$
STZ+E.E1	$0.23+0.05^{a}$	$3.11+0.81^{d}$	$1.80+0.33^{c}$
STZ+E.E2	$0.30+0.03^{ab}$	$2.43+0.22^{c}$	$1.16+0.19^{b}$
STZ+STD DRGS	$0.52+0.14^{c}$	$1.04+0.25^{ab}$	$0.57+0.15^{a}$

Values are represented as means +SD (n=6). Values carrying different superscript letters along the column shows significant difference ($p<0.05$).NC- Normal control, DC-Diabetic control, STZ-Streptozotocin, AE1-Aqueous extract(100mg),Aqueous extract(200mg), EE1-Ethanol extract(100mg), EE2-Ethanol extract(200mg), STD DRGS- Sildenafil citrate(5mg) + Metformin (100mg).TC-Total cholesterol: TG- Triglyceride: HDL-High density lipoprotein cholesterol: LDL-Low density lipoprotein cholesterol. NB: Values of HDL-c/TC ratio < 0.30& LDL-c/HDL-c ratio >2.3 are atherogenic and undesirable (Quispe *et al.*, 2020).

Hormonal and Sperm parameters.

The root bark extracts of *Feretia apodanthera* in normal and STZ induced diabetic rats at 100 and 200mg/kg bw on day, 14 and 21 shows significant($p<0.05$) difference on the serum testosterone content of the animals compared to the untreated control animals (Table 5.0). However, during the first week of treatment, the extracts treated groups presented no significant difference ($p>0.05$) except for aqueous extract (200mg). At the end of experimental period, the highest dose group of the extract (200mg/kg body weight) of aqueous extract had increased the testosterone concentration in the serum of the animals by 1.15-fold compared to the negative control. Furthermore, table 6.0 shows the effect of the root bark extract of *Feretia apodanthera* on sperm parameters. The sperm count, motility and viability were significantly ($p<0.05$) decreased in diabetic control rats compared to the other groups. Though ethanol and aqueous extracts had dose dependent effects on sperm parameters. However, treatment with 200mg/kg b.w aqueous extract significantly ($p<0.05$) increased sperm count, motility and viability compared to other treated groups.

Table 5.0: Effect of root bark extracts of *Feretia apodanthera* on Serum Testosterone concentrations of Diabetic Male rats.

TREATMENT GROUP	Day 7	Day 14	Day 21
N.C	5.35 ± 0.54^{e}	5.23 ± 4.41^{f}	5.72 ± 0.28^{d}
D.C	1.62 ± 0.21^{a}	1.72 ± 0.21^{a}	2.62 ± 0.31^{a}
STZ+AE1	3.03 ± 0.42^{b}	3.18 ± 0.38^{bc}	3.57 ± 0.94^{b}
STZ+AE2	4.07 ± 0.33^{c}	4.17 ± 0.33^{d}	4.57 ± 0.19^{c}
STZ+EE1	2.97 ± 0.29^{b}	3.13 ± 0.28^{b}	3.38 ± 0.45^{b}
STZ+EE2	3.35 ± 0.32^{b}	3.53 ± 0.32^{c}	3.75 ± 0.71^{b}
STZ+STD DRG	4.52 ± 0.15^{d}	4.60 ± 0.18^{e}	5.00 ± 0.55^{c}

Values are represented as means +SD (n=6). Values carrying different superscript letters along the column shows significant difference ($p<0.05$).NC- Normal control, DC-Diabetic control, STZ-Streptozotocin, AE1-Aqueous extract(100mg), Aqueous extract(200mg), EE1-Ethanol extract(100mg), EE2-Ethanol extract(200mg), STD DRGS- Sildenafil citrate(5mg) + Metformin (100mg).

Table 6.0: Effect of extracts of root bark of *Feretia apodanthera* on Sperm parameters in STZ induced Diabetic Male Rats

Group	COUNT($\times 10^6$/ml)	MOTILITY (%)	VIABILITY (%)
NC	26.33 ± 1.52^{e}	68.35 ± 1.41^{f}	83.42 ± 1.90^{g}
DC	8.32 ± 1.15^{a}	21.22 ± 1.26^{a}	30.58 ± 1.96^{a}
STZ+A.E1	15.07 ± 2.57^{c}	38.02 ± 0.97^{c}	40.00 ± 2.33^{c}
STZ+A.E2	22.77 ± 1.58^{d}	47.42 ± 1.28^{d}	73.08 ± 2.65^{e}
STZ+E.E1	11.68 ± 1.18^{b}	28.70 ± 1.79^{b}	33.77 ± 2.40^{b}
STZ+E.E2	14.85 ± 0.91^{c}	36.95 ± 1.97^{c}	47.98 ± 2.39^{d}
STZ+STD DRGS	25.10 ± 1.31^{e}	60.43 ± 1.37^{e}	77.92 ± 2.20^{f}

Values are represented as means +SD (n=6). Values carrying different superscript letters along the column shows significant difference ($p<0.05$).NC- Normal control, DC-Diabetic control, STZ-Streptozotocin, AE1-Aqueous extract(100mg), Aqueous extract(200mg), EE1-Ethanol extract(100mg), EE2-Ethanol extract(200mg), STD DRGS- Sildenafil citrate(5mg) + Metformin (100mg).

Histopathology of the Testes

The normal control group's tissue samples, when examined histologically, displayed typical characteristics of the spermatogenic cells together with a healthy glance of the seminiferous tubules (with normal gap between the basement membrane and spermatogonia). On the other hand, there is substantial atrophy in the diabetic control group, which results in thicker basement membrane and smaller seminiferous tubule diameter. Pyknosis and severe necrosis were also visible. The histological section of the testes of STZ-induced diabetic male rats treated with ethanolic and aqueous root bark extracts of *Feretia apodanthera* for 21 days shows lesser differences observed except for the aqueous extracts treated with 200 mg/kg b.w, which demonstrate certain aspects of the basement membrane linked to the appearances of sertoli cells along with spermatogonia, these features werelike those observed in the healthy control group (Figure 2.0). In comparison to the healthy control group, the conventional medication combination (metformin with sildenafil citrate) does not dramatically alter the basement membrane's appearance. However, apoptotic cells were detected in this group as well, albeit in much smaller quantities than in the untreated diabetes group.

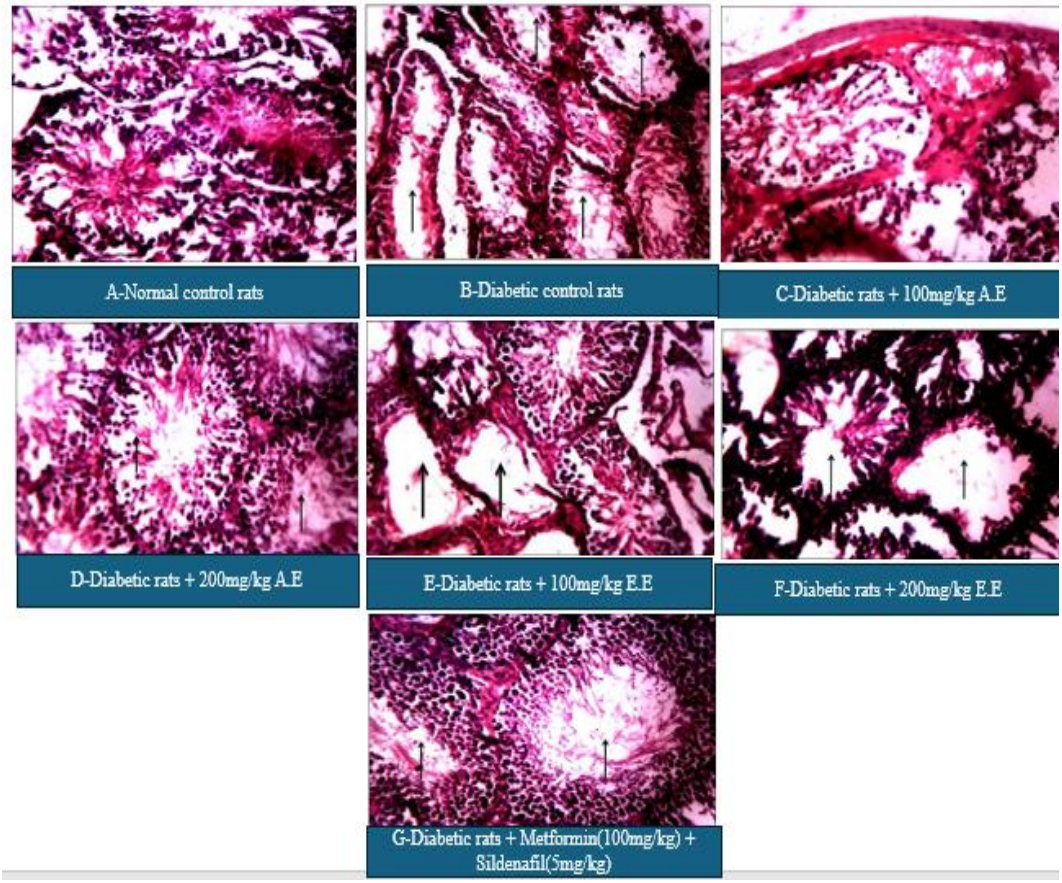


Figure 2.0: Histopathological Assessments of the Testicular tissue sections stained using hematoxylin and eosin (H&E x250) in the different experimental groups. A=Normal features of spematogenic cells; B=Intense necrosis and pyknosis of spermatogenic cells: C=Moderate necrosis of spermatogenic cells: D=Slight necrosis of the spermatogenic cells with testicular hardening: E= Deep necrosis of the spermatogenic cells: F= Deep necrosis and pyknosis of the spermatogenic cells: G=relatively healthy spermatogenic cells. A.E-Aqueous extract of Feretia apodanthera & EE-Ethanol extracts of root Bark extracts of Feretia apodanthera.

Discussion

1,1-diphenyl-2-picrylhydrazyl (DPPH), a free radical that remains stable at ambient temperature, transforms into a stable diamagnetic molecule by accepting an electron or hydrogen donation. In this present study, the concentration of each extract needed to scavenge 50% of the DPPH radical present in the assay medium was determined through analyzing the extracts of *Feretia apodanthera* over a range of dilutions. This is known as half-maximal inhibitory concentration (IC_{50}), which is defined as the concentration of antioxidants required to scavenge 50% of the DPPH present in the test solution. Better DPPH radical scavenging activity is indicated by a lower IC_{50} value (Ereifej *et al.*, 2016).The outcome of this studydemonstrates that the aqueous extract had the highest antioxidant capacity amongst the root bark extracts of *Feretia apodanthera*, though it differed significantly ($p<0.05$) from vitamin C (Standard).Due to its lower IC_{50} value, aqueous extract has the strongest antioxidant activity among all the tested extracts, followed by ethanol and n-hexane extracts respectively. This outcome is consistent with a prior study conducted by Coulibaly *et al.* (2014), which found that aqueous extract of *Feretia apodanthera* had a stronger antioxidant potential. A little inconsistency was noted in the research conducted by Owolabi *et al.* (2014), wherein it was observed that the antioxidant power of ethanol root bark extracts of *Feretia apodanthera* was considerably greater than that of the aqueous extract. This might be due to different extraction

techniques employed. The results of this study show that diabetes significantly ($p<0.05$) reduces the wistar rats' body weight. This is consistent with the findings of Heidari *et al.* (2021). However, the body weight was significantly ($p<0.05$) increased by the extracts, particularly the 200 mg/kg bw aqueous root bark extract of *Feretia apodanthera*.

Table 2.0 shows that, in streptozotocin induced untreated diabetic rats, there are characteristics of dyslipidemia as evidenced by decrease in high-density lipoprotein (HDL) and increases in total cholesterol, plasma triglycerides, and low-density lipoprotein (LDL) cholesterol. Lipids are indispensable in the pathophysiology of diabetes mellitus. Diabetes is linked to significant changes in triglycerides, plasma lipids and lipoproteins as well as a greater likelihood of cardiovascular disease (Wang *et al.*, 2018). The two most prevalent lipid dysfunctions in diabetes are hypertriglyceridemia and hypercholesterolemia. Under normal physiological conditions, the elevated level of insulin facilitates the uptake of fatty acid into the adipose tissue, increased synthesis of triglycerides and suppression of lipolysis (James *et al.*, 2020). Insulin also triggers lipoprotein lipase and breakdown of triglycerides. However, when insulin is deficient, there is a reduced activity of lipoprotein lipase with a concomitant increase in lipolysis, thereby resulting to dyslipidemia, this is evident in diabetic conditions (Amaechi *et al.*, 2015).

Administration of aqueous and ethanol extract of *Feretia apodanthera* improves the level of lipid profiles, with aqueous extract 200mg/kg b.w showing significant($p<0.05$) increase in HDL-c and decrease in total cholesterol, low-density lipoprotein cholesterol and plasma triglycerides.As a result of its many antiatherogenic properties, HDL-c is believed to have an inverse relationship with cardiovascular disease (Farbstein and Levy 2012).In subjects with poorly managed diabetes, enhancement in glycemic management may raise blood HDL-c levels and significantly reduce triglyceride and LDL-c levels. Our previous work has reported the antidiabetic profile of *Feretia apodanthera* in streptozotocin induced diabetic rats. In this present work, the diabetic untreated group shows significant ($p<0.05$) decrease in the level of testosterone. These changes could be attributed to damage of the Leydig cells (Heeba and Hamza 2015). Conversely, the administration of *Feretia apodanthera* root bark extracts significantly boost testosterone levels, which may enhance sexual function by acting as an antagonist of gamma aminobutyric acid (GABA), through the steroidal precursor dehydroepiandrosterone. The enhanced sexual function may be due to the conversion of dehydroepiandrosterone to testosterone and its metabolite, even though the precursor was not evaluated in this study (Khaneshi *et al.*, 2013 & Heidari *et al.*, 2021).

The correlation between diabetes and sperm characteristics has produced inconsistent findings for researchers. There has been no discernible change in the number, motility, and viability of sperm in human subjects with diabetes mellitus (Agbaje *et al.*, 2007 & Petroianu *et al*, 2009). Nonetheless, research employing an animal model has revealed a notable variation in sperm characteristics. The supplementation of root bark aqueous extract of *Feretia apodanthera* in this study significantly ($p<0.05$) altered sperm motility, count, and viability. The outcome is consistent with earlier research conducted in mice and rats, respectively, by Magoli *et al.* (2013) and Arikawe *et al.* (2006). It appears from clinical and experimental evidence that sperm parameters are impacted in diabetes mellitus situations, particularly in animal experiments. Additionally, it was proposed that the onset of these modifications is caused by interplay of hormonal fluctuations, neuropathy incidence, and oxidative stress (He *et al.*, 2021).In the histology of the testes, it was observed that streptozotocin induction leads to damage in testicular morphology and alteration of spermatogenesis. The damage could be prompted by the toxic effect of streptozotocin, leading to decrease in testosterone levels linked with deficiency or reduced levels of insulin, since insulin could potentially regulate apoptotic

germ cell death and male infertility induced by diabetes. Treatment with 200mg root bark aqueous extract of *Feretia apodanthera* shows lesser thickening of the seminiferous tubule's basement membrane as well as enhanced sperm production. Furthermore, antioxidants may play a crucial role in the mitigation of testicular dysfunction in diabetes since low androgenic hormones and oxidative stress are implicated in disruption of spermatogenesis (Huang, 2024).

In conclusion, the current data point to the strong antioxidant potential of root bark extracts of *Feretia apodanthera*, both aqueous and ethanol, and their ability to enhance lipid profiles, testosterone levels, and sperm parameters (count, motility, and viability) in STZ-induced diabetic rats. Furthermore, testicular damage and sperm parameters appear to respond favorably to the greater dose of aqueous extract (200 mg/kg b.w).

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