



## ***In vivo* Studies on the Phytotherapeutic and Fertility Effects of *Dracaena Arborea* Extract in Alloxan-induced Type 1 Diabetic Male Rats**

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### **Authors' contributions**

This work was carried out in collaboration between all authors. Author OSO participated in study design, data collection, and interpretation, contributed to drafting/ revising of manuscript. Author SOO participated in study design, data analysis and interpretation, did literature search and drafted/edited/revised manuscript, and serve as the corresponding author. Author JOO participated in study design, data interpretation and revising of manuscript. Authors SKL and OAA participated in study design and participated in data collection. All authors read and approved the final manuscript.

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### **ABSTRACT**

**Aim:** This study was designed to explore and exploit the phytotherapeutic and fertility effects of ethanolic leaf extract of *Dracaena arborea* in type-1 Alloxan-induced diabetic rats. The phytotherapeutic effects of *Dracaena arborea* on hematological parameters, appetite, spermiogram, histological architecture and histomorphometrics (stereology) of testicular and/or pancreatic tissues of treated and untreated rats were carried out.

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**Place and Duration of Study:** Department of Anatomy, College of Medicine, Lagos State University, Ikeja, Lagos, Nigeria, between October, 2012 and February, 2013.

**Methodology:** 24 healthy normal male rats were recruited for this study. They were divided into four groups in a randomized trial; with group A consisting of non-diabetic healthy rats that received only the vehicle (0.5 mg/kg of 2% acacia solution); while group B, C & D was injected intraperitoneally with a single dose of Alloxan monohydrate (ALX) at 100 mg/kg prior to DAE treatment. Groups C and D were subsequently administered DAE orally 72 hours post administration of ALX, at daily doses of 100 mg/kg and 300 mg/kg respectively.

**Results:** ALX (100 mg/kg) was found to induce type 1 diabetic conditions in the rats, demonstrated by the significant increase ( $P < 0.05$ ) in the glucose levels, and a decline in appetite (water and food intakes). Conversely, administration of DAE at 100 and 300 mg/kg revealed significant dose and time- dependent increase ( $P < 0.05$ ) in glucose tolerance and appetite (water and food intakes) in DAE treated groups compared to the untreated or ALX treated only group. Significant normalization ( $P < 0.05$ ) of red blood cell count, packed cell volume and Hemoglobin levels were also observed in diabetic rats treated with the 100 mg/kg and 300 mg/kg DAE. In addition, testicular and pancreatic histopathological profiles of both DAE treated groups show evidences of appreciable normalization of ALX-induced pathology.

**Conclusion:** Our findings indicates that DAE may offer great therapeutic benefit in the treatment of type-1 diabetes mellitus and normalizing testicular dysfunction or infertility in diabetic patients.

**Keywords:** *Diabetes mellitus; testicular dysfunction; Dracaena arborea; alloxan monohydrate; hyperglycemia; reactive oxygen species; oxidative stress.*

## ABBREVIATIONS

DAE: *Dracaena arborea* (leaf) Ethanollic Extract;

ALX: Alloxan Monohydrate;

SHBG: Sex Hormone Binding Globulin;

EC<sub>50</sub>: Effective Dose (50%);

LD<sub>50</sub>: Lethal Dose (50%);

β-cells: Beta-Cells;

α-cells: Alpha Cells;

ROS: Reactive Oxygen Species

## 1. INTRODUCTION

Diabetes mellitus is a chronic and metabolic public health disease that affects all age groups, projected to become the 7<sup>th</sup> leading cause of death by 2030, with more than 80% of the deaths occurring in the low- and middle- income countries [1]. In Africa, Nigeria with a population of about 173.6 million has the greatest number of people living with diabetes, with an estimated burden of about 1.7 million currently living with the disease and about 3.85 million people estimated to have impaired glucose tolerance [2]. The incidence of diabetes in Nigeria have been projected by the World Health Organization (W.H.O) to rise to about 4.8 million by 2030 [3].

In men, sexual disorders such as impotency, low libido and impaired spermatogenesis are well-recognized consequences of diabetes mellitus [4,5]. The presence of neuro-endocrine dysfunctions in impotent diabetic men and also in

diabetic murine models have been reported previously [6,7]. Low testosterone and sex hormone binding globulin (SHBG) concentrations in elderly men have also been demonstrated to be associated with established diabetes [6]. Other symptoms that have been documented include: testicular atrophy and hypogonadism with desquamation of the germinal epithelium [8-10]. Testicular biopsies from oligospermic or impotent men with diabetes mellitus reveal discrete ultrastructural lesions in the apical sertoli cell cytoplasm, spermatogenic disruption and morphological changes in the interstitial compartment indicating microvascular complications [11]. In addition, altered testicular structure and functions have been observed in diabetic animal models [12].

However, since modern treatment options available for the regulation of testicular function anomalies induced by diabetes are not readily available for patients in developing countries, and also becoming unaffordable, and often leads to more serious side- effects; attention has been shifted towards alternative therapies such as traditional herbal medicine and phytotherapy [13,14]. In Nigeria, *Dracaena arborea* plant extracts have been locally used as aphrodisiac to treat sexual inadequacy and to stimulate sexual vigor [15]. On the basis of the above mentioned history, we hypothesized that ethanollic leaf extract of *Dracaena arborea* may have potent phytotherapeutic prospect for the symptomatic

treatment of sexual dysfunction in diabetic male patient and that this potency may be due to the phytochemical constituents of this plant extracts. The present study was undertaken to investigate the phytotherapeutic and fertility effects of the leaf extract of the aphrodisiac plant, *Dracaena arborea*, on blood glucose level, appetite, testicular morphology, spermogram, histopathology and histomorphometrics of the pancreas and/or testes in alloxan-induced type 1 diabetic Sprague dawley rats.

## 2. MATERIALS AND METHODS

### 2.1 Plant Collection

The *Dracaena arborea* was harvested from a deciduous forest in Ogbomosho, Nigeria. Identification and characterization of the plant was done at the Department of Botany, University of Lagos, Nigeria under the voucher specimen number DSN: #69. Plant's botanical name was also checked and verified at <http://www.theplantlist.org>.

### 2.2 Preparation of Leaf Extract of *Dracaena arborea* (DAE)

Fresh leaves of *D. arborea* were collected, washed and shade-dried at room temperature for 7 days after which they were grounded into a fine powder. About 650 grams of the pulverized leaves of *D. arborea* was soaked in 6500 ml of 95% ethyl alcohol in 3 cycles using Soxhlet extractor and left for 24 hours. The crude extract was filtered using filter paper (Whatman No 4), concentrated and dried in a rotary vacuum evaporator at 30°C to obtain a 97.2 g dry residue (a viscous brownish-colored extract (14.9% vol.)). The ethanolic leaf extract was stored in an air tight bottle kept in a refrigerator at 4°C pending use.

### 2.3 Induction of Type-1 Diabetic Condition

Diabetic condition was induced experimentally in rats fasted 12 hours prior to injection with a single dose of 100 mg/kg Alloxan monohydrate (ALX, Sigma, sigma, St. Louis, MO, USA) dissolved in normal saline and administered intraperitoneally [10]. Two hours after the administration of ALX, all rats were given an intra-peritoneal injection of glucose 5% in order to overcome ALX-induced hypoglycemia due to the destruction of the pancreatic  $\beta$ -cells and massive release of insulin. Fasting blood glucose

level (diabetic status) was measured 48 hours after ALX treatment using one Touch Ultra Mini Glucometer (Life Scan Inc. Milpitas, CA, USA) with a drop of blood obtained by tail-vein puncture. Rats with blood glucose values of  $\geq 150$  mg/dl were considered diabetic.

### 2.4 Phytochemical Screening of DAE

Phytochemical screening was carried out on the leaf extracts of *Dracaena arborea* using the method of Trease and Evans [16], which gives us baseline information of the possible active phytochemicals present in the extracts. Coarse powder of the leaves was extracted with absolute ethanol for 24 hours. The filtrates were thereafter concentrated in vacuum at 40°C.

**Alkaloids Assay:** Thin layer chromatography method described by Farnsworth and Euler [16] was used.

**Saponins Assay:** Frothing and blood hemolysis test was used [16].

**Antraquinones Assay:** This was assessed with Borntrager's test [16].

**Glycosides Assay:** Series of tests, Salkowski, Lieberman, Keller-Kilani, Legal and Keddes as described by Trease and Evans [16] were used.

**Flavonoid Assay:** Lead acetate, ferric chloride and sodium hydroxide tests were used [16].

**Tannin Assay:** Ferric chloride test was used for the assessment [16].

Other chemical substances screened for include: cyanogenic glycosides and reducing compounds [16].

### 2.5 DAE Acute Toxicity Test

The acute toxicity of ethanolic leaf extract of *Dracaena arborea* was determined by using thirty-five (35) male Sprague Dawley rats (160-180 grams) which were maintained under the standard conditions. The animals were randomly distributed into a control group and six treated groups (I-VI), containing five animals per group. After depriving them food for 14 h prior to the start of the experiment with only access to water, the control group was administered single dose of 0.5 mg/kg of 2% acacia solution orally. In like manner, each treated groups were administered single dose of ethanolic leaf extract

of *Dracaena arborea* orally at doses of 1.0, 2.5, 5.0, 10, 15 and 20.0 g/kg body weight respectively. They were closely observed in the first 4 hours and then hourly for the next 12 hours followed by hourly intervals for the next 2 weeks after the drug administration to observe for any death or display of any abnormal physiological, behavioral or neurological signs [17,18]. The median lethal dose (LD<sub>50</sub>) was estimated for each group by Log dose – Probit analysis [19].

## 2.6 Experimental Design

Twenty four healthy male Sprague dawley rats weighing between 160-180 g were obtained from the Laboratory Animal Center of the College of Medicine, Lagos State University, Nigeria. They were placed in clean cages under a 12 hour light: dark cycle, 50% humidity at 26±2°C with standard food and water ad libitum in a well-ventilated room in which they were allowed to acclimatize to laboratory condition for a period of two weeks prior to start of experiment. Study was approved by the ethical committee of the Lagos State University, Nigeria in conformity with the International, National and Institutional guidelines for the care of laboratory animals in biomedical research and use of laboratory animals in biomedical research as promulgated by the Canadian Council of Animal Care and the guiding principles for research involving animals as recommended by the declaration of Helsinki and the guiding principles in the care and use of animals [20].

## 2.7 Animal Grouping

The twenty four (12 to 14 weeks old) Sprague dawley rats weighing between 190 - 220 g were used for this study. After confirmation of diabetes in Alloxan injected rats. The rats were randomly divided into 4 groups consisting of six rats each and were treated as follows;

- Group A: Healthy normal male rats given only vehicles (0.5 ml/ kg body weight of 2% acacia solution), which served as control group.
- Group B: Alloxan monohydrate-induced diabetic rats given 0.5 ml/kg body weight/day of 2% acacia solution (Diabetic control group).
- Group C: Diabetic rats treated with 100 mg/kg body weight/day of *Dracaena arborea* leaf extract.
- Group D: Diabetic rats treated with 300 mg/kg of body weight/day of *Dracaena arborea* leaf extract.

The vehicle (2% acacia solution) and the leaf extracts were administered per Os once daily for the entire period of 8 weeks with the aid of an endogastric tube. The EC<sub>50</sub> at 300 mg/kg of DAE from our preliminary studies was used as the therapeutic dose for the experiment. Rats were maintained in these treatment regimens for eight weeks with free access to food and water *ad libitum*. Body weight was measured per week for the entire eight weeks.

## 2.8 Assessment of Weekly Changes in Serum Glucose Levels

The serum glucose level was estimated by the glucose oxidase method using a commercially available diagnostic kit, (ACCU-CHEK, Roche Diagnostics, USA). Serum samples were collected each week for blood glucose analysis from rats in each group via the tail vein. Rats were carefully restrained and the tail vein pinched with a sterile needle to get blood for serum glucose analysis.

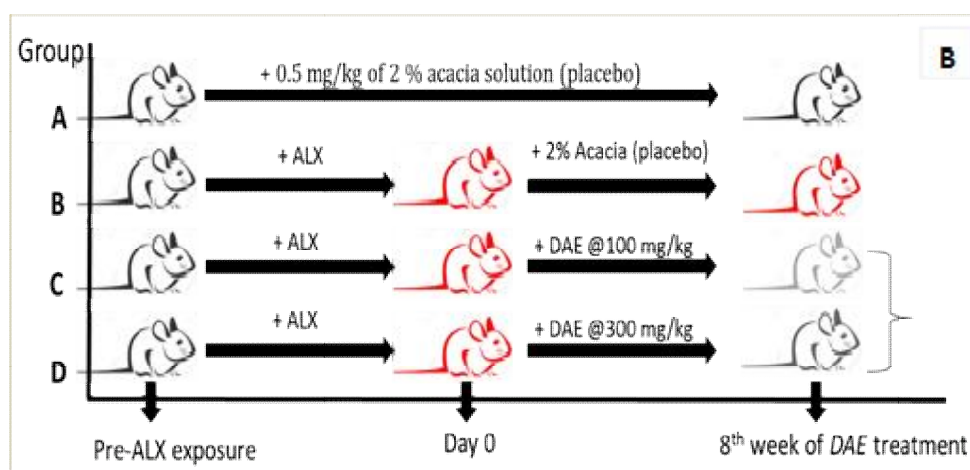
## 2.9 Tissue Preparation and Spermogram Assessment

Eight weeks post treatment; rats were sacrificed under chloroform anesthesia. The testis and epididymis of each rat after scrotal excision were immediately collected and washed with ice-cold physiologic saline solution (0.9%, w/v), blotted and weighed. For evaluation of sperm abnormalities and sperm-shape analysis, the epididymis was excised and minced in physiological saline. The tail of the epididymis was cut into small pieces in normal saline. Briefly the smears of sperm suspension were made on clean glass slides and stained with methylene blue according to the [21]. The stained smears were observed under a light microscopic with 40X objective. Sperms were classified into normal and abnormal. At least 1000 sperm cells per animal were assessed for morphological abnormalities. The total sperm abnormality was expressed as percentage incidence. The sperm abnormalities were evaluated according to standard method of [22].

## 2.10 Testicular Morphology and Geometry

### 2.10.1 Determination of histomorphometric parameters

For each testis, seven “vertical sections” from the polar and the equatorial regions were sampled [23] and an unbiased numerical estimation of the



**Fig. 1a & b. Picture of *Dracaena arborea* plant and the in vivo study treatment plan respectively**

Fig. 1. Picture of *Dracaena arborea* tree showing the green colored leaves from which the extract was isolated (Inlet: showing a young *D. arborea* plant without a shoot, showing the bright green colored leaves extract).

Fig. 1b: A diagrammatic sketch showing the treatment plans and methodology for this study. The rats were grouped into group A-D, with each group made up of 6 rats. The red colored rats at Day 0 of treatment are used to depict type 1 diabetic rats due to pre-exposure to Alloxan monohydrate. The treatments with DAE (100 and 300 mg/kg) were maintained daily in group C and D for 8 consecutive weeks

following morphometric parameters were determined using a systematic random scheme [24]; testicular volume and weight; diameter (D) and cross-sectional area of the seminiferous tubules ( $A_C$ ), number of profiles of seminiferous tubules per unit area of testis ( $N_A$ ), and numerical density of the seminiferous tubules ( $N_V$ ). Seven "vertical sections" per testis were selected by a systematic sampling method that ensured fair distribution between the polar and equatorial regions of each testis. Briefly, a section was taken at the equator of each testis; one on each side of the equator, three-quarters of the distance between the pole and the equator; another half-way between each pole and the

equator; and one on each side of the equator, a quarter of the distance from each of the pole. For each stereological parameter ( $D$ ,  $A_C$ ,  $N_A$  and  $N_V$ ), five randomly selected fields from all the seven sections of a single testis were viewed, and estimation on each carried out. The average from a total of seventy readings from five fields in seven sections of the two testes of one rat was obtained and this was recorded as one observation. The evaluation of the diameter was done with calibrated eyepiece and stage grids mounted on a light research microscope at 100 X magnification. Estimation of volume or density of testicular components and the number of seminiferous tubules were done appropriately.

1. Diameter ( $D$ ) of seminiferous tubules: The diameter of seminiferous tubules with profiles that were round or nearly round were measured for each animal and a mean,  $D$ , was determined by taking the average of two diameters,  $D_1$  and  $D_2$  (Perpendicular to one another).  $D_1$  and  $D_2$  were taken only when  $D_1/D_2 \geq 0.85$ .
2. Cross-sectional area ( $A_C$ ) of the seminiferous tubules: The cross-sectional areas of the seminiferous tubules were determined from the formula  $A_C = \pi D^2/4$ , (where  $\pi$  is equivalent to 3.142 and  $D$  the mean diameter of the seminiferous tubules).
3. Number of profiles of seminiferous tubules in a unit area of testis ( $N_A$ ): The Number of profiles of seminiferous tubules per unit area was determined by using the unbiased counting frame proposed by Gundersen [20]. Using this frame, in addition to counting profiles completely inside the frame we counted all profiles with any part inside the frame provided they did not touch or intersect the forbidden line (full-drawn line) or exclusion edges or their extension.
4. Numerical Density ( $N_V$ ) of seminiferous tubules: This is the number of profiles per unit volume and was determined by using the modified Floderus equation:  $N_V = N_A/(D + T)$  [23].

Where,  $N_A$  is the number of profiles per unit area,  $D$  is the diameter and  $T$  the average thickness of the section.

#### **2.10.2 Histological analysis of pancreatic and testicular tissues**

This was done as described by [25]. Briefly, the organs were removed from bouin's fluid after 48 hours and fixed in fresh bouin's fluid for another 72 hours. Each testis was sliced into slabs of about 0.5 cm thick and dehydrated in varying degree of alcohol (70%, 90%). From 90% alcohol to 3 changes of absolute alcohol for 1 hour each, then into chloroform for about 10 h and later transferred into fresh chloroform for about 30 minutes. The tissues were placed in 3 changes of molten paraffin wax for 30 minutes each in an oven at 57°C. They were thereafter placed vertically in molten paraffin wax inside a plastic mold and left overnight to cool and solidify. They were later trimmed and mounted on wooden blocks. Serial sections were cut using a rotary microtome at 5  $\mu$ m thickness. Sections were

floated in a water bath and picked by albuminized slides and dried on the hot plate at 57°C. For staining of sections, the slides were de-waxed in staining racks and placed in staining wells containing xylene and rehydrated in varying degree of alcohol (absolute, 90%, and 70%) and then to water for 5 minutes after which they were stained with hematoxyline for 3 minutes. Excess hematoxyline was washed off with water and differentiated with 1% acid alcohol. Sections were thereafter rinsed under running tap water and then left for 5 minutes for bluing. Sections were counterstained with 1% eosin and washed off with water. They were dehydrated with 70%, 90% and absolute alcohol and cleared in xylene to remove all traces of water. A drop of mountant was placed on the surface of the slide and covered with a 22 by 22 cm<sup>2</sup> cover slip. Light microscopy was used for the evaluations and the photomicrographs were taken. The pancreatic tissue sections at 5 $\mu$ m were stained with aldehyde fuchsin [26].

#### **2.11 Statistical Analysis**

All data were expressed as mean  $\pm$  standard error of means (SEM). Data were also statistically analyzed using one-way ANOVA followed by Student t-test analysis to test the level of significance and difference between the results of the treated, untreated and control groups. The differences were considered statistically significant at  $P < 0.05$ . Graph Pad prism program (version 5) was used as analyzing software and graphs plotted using Microsoft Excel v. 2013 Program.

### **3. RESULTS**

#### **3.1 Assessment of Acute Toxicity Test (LD50)**

The acute toxicity study result (Table 1), showed that 4 out of the 5 animals that received 20.0 g/kg body weight of the extract died within 4 hours (80% death) while the animals that received 2.5 g/kg body weight survived beyond 24 h. The LD<sub>50</sub> of the drug was therefore calculated to be 15 g/kg body weight. The LD<sub>50</sub> of the extract was determined by plotting a graph of Probit on the Y-axis against the Log dose on the X-axis.

#### **3.2 Qualitative Phytochemical Analysis**

Phytochemical investigations were carried out on the leaf extract of *Dracaena arborea*. The result of the qualitative analysis of *Dracaena arborea* is

presented in Table 2. The medicinal value of this plant extract lies in some bioactive phytochemical compounds that have definitive or modulatory physiological activities on the body. The active compounds found in DAE from our preliminary phytochemical screening study include: alkaloids, saponins, terpenoids compounds, anthraquinones, flavonoids, tannins, cardiac glycosides and reducing sugar.

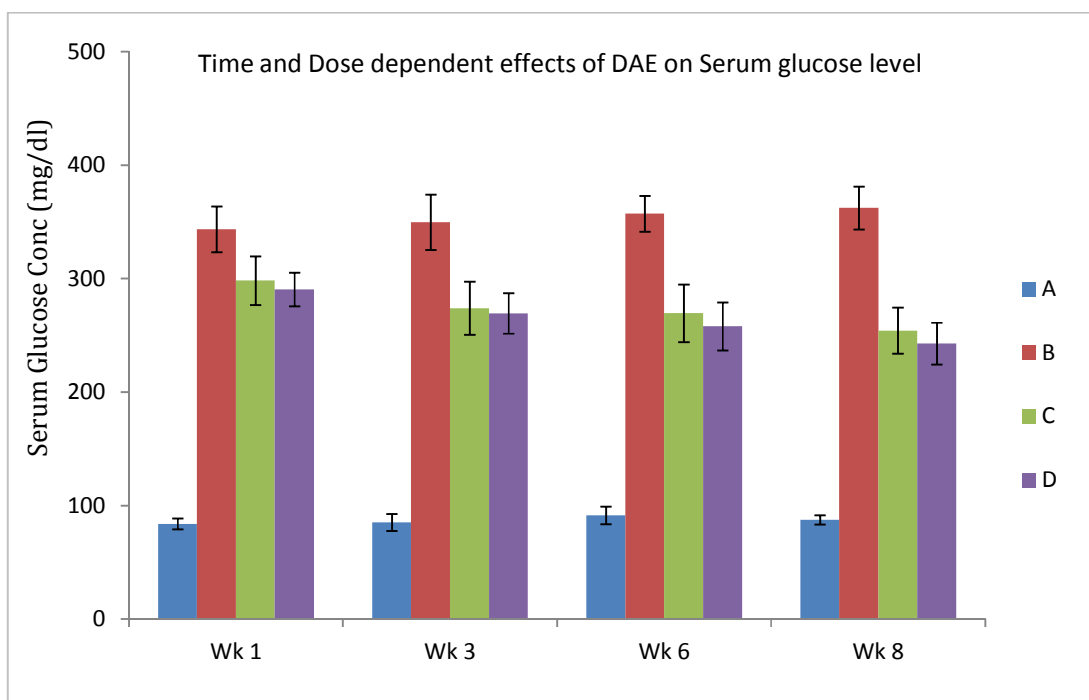
### 3.3 Effects of DAE on Serum Glucose Concentration

A significant increase ( $P < 0.05$ ) in serum glucose concentration (mg/dl) was observed in ALX treated rats, most especially in diabetic control group (B) after on a weekly basis as recorded for week 1, 3, 6 and 8, relative to the

normal control group (Fig. 2). The weekly mean serum glucose concentrations for the DAE treated groups of rat (C & D) significantly decreased ( $P < 0.05$ ) toward normal for the 8 weeks of the study.

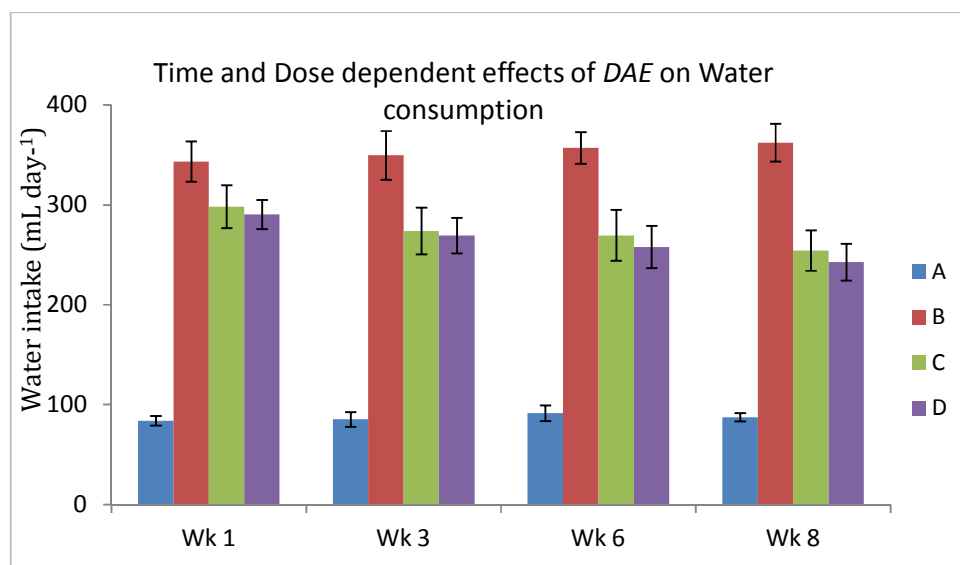
### 3.4 Effects of DAE and ALX on Appetite

The results in Figs. 3 and 4 indicate that water and food intake respectively increased significantly ( $P < 0.05$ ) after administration of ALX in male rats. When compared with the diabetic control (group B), there were significant decrease ( $P < 0.05$ ) in the water and food intake following DAE administration in group C and D. No significant statistical difference in the food and water consumption rate was observed between group C and D.



**Fig. 2. Effects of oral administration of ethanolic leaf extract of *D. arborea* on serum glucose concentration (mg/dl) in ALX-diabetic male rats for a period of eight weeks post treatment**

Graph showing the time and dose dependent effect of DAE following ALX-induced diabetic condition in rats of between group A and B, and group C and D. Rats were grouped into 4 comprising of normal control (A), diabetic control (B), diabetic rats treated with 100 mg/kg of DAE and lastly diabetic rats treated with 300 mg/kg of DAE. After induction of diabetes in group B, C and D with ALX, The serum glucose level was estimated by the glucose oxidase method using a commercially available diagnostic kit, (ACCU-CHEK, Roche Diagnostics, USA). Serum samples were collected each week for blood glucose analysis and recorded for week 1, 3, 6, and 8 with a drop of blood obtained by tail-vein puncture. Values are represented as the mean  $\pm$  SEM for the 6 rats per group. a: Statistically significant when compared to control group (A) at  $P < 0.05$ ; b: Statistically significant when compared to diabetic untreated group (B) at  $P < 0.05$



**Fig. 3. Effects of oral administration of *Dracaena arborea* extract for 8 weeks on water intake (ml/day) in ALX-diabetic male rats**

Graph showing the time and dose dependent effects of DAE treatments on water intake rate (appetite) following ALX-induced diabetic condition in rats of between group A and B, and group C and D. Rats were grouped into 4, comprising of normal control (A), diabetic control (B), diabetic rats treated with 100 mg/kg of DAE and lastly diabetic rats treated with 300 mg/kg of DAE. Values are represented as the mean  $\pm$  SEM for the 6 rats per group.

a: Statistically significant when compared to control group (A) at  $P < 0.05$ ; b: Statistically significant when compared to diabetic untreated group (B) at  $P < 0.05$

**Table 1. Acute toxicity test for Ethanolic leaf extract of *Dracaena arborea* in sprague dawley rats**

Group	Dose (g/kg)	Log dose	Mortality	% Mortality	Probit
I	1.0	3.00	0/5	0.0	0.0
II	2.5	3.40	0/5	0.0	0.0
III	5.0	3.70	1/5	20.0	4.2
IV	10.0	4.00	1/5	20.0	4.2
V	15.0	4.18	2/5	40.0	4.7
VI	20.0	4.30	4/5	80.0	5.8

### 3.5 Effects of DAE and ALX on Hematological Parameters

Appreciable significant normalization ( $P < 0.05$ ) of the red blood cell counts (RBC), packed cell volume (PCV) and hemoglobin levels (Hb) were also observed in the diabetic rats treated with the 100 mg/kg and 300 mg/kg of DAE (Table 3). There was also a slight rise in the mean leukocyte counts (WBC) of the treated groups (C & D). No significant difference ( $P > 0.05$ ) in mean corpuscular hemoglobin concentration (MCHC) for the treated animals compared to the control. There was also no statistically significant difference ( $P > 0.05$ ) in mean corpuscular volume (MCV) of all groups.

### 3.6 Effects of DAE on Testicular Parameters and Spermogram

There was dose dependent statistically significant increase ( $P < 0.05$ ) towards normal in the testicular and epididymal weights of rats in group C and D. ALX caused a sharp significant fall ( $P < 0.05$ ) in testicular weight, epididymal weight, sperm motility, and percentage of sperms with normal morphology while increasing the percentage of cells with abnormal morphology in group B (diabetic control) as observed in Table 4. These parameters were significantly reversed ( $P < 0.05$ ) dose-dependently in DAE treated group of rats (C & D).



**Table 2. Major Phytochemicals screened for and identified in *D. arborea* leaf extract**

Phytochemical	Test	Observation	Inference
Alkaloids	Dragendorff's test	Cloudy orange precipitate	Alkaloid present
	Wagner's test	Dark brown precipitate	Alkaloid present
	Mayer's test	Yellow color precipitate	Alkaloid present
	Dragendorff's confirmatory test on TLC	Dark color spotted on TLC	Alkaloid Confirmed
Saponins	Benedict's test	Reddish brown precipitate	Reducing sugar present
	Frothing test	Foam persist after 15 minutes	Saponin present
	Emulsion test Hemolysis test	Stable emulsion obtained Hemolysis in tubes with extract	Saponin present Saponin confirmed
Anthraquinones	Borntrager's test	Red color precipitate	Free anthraquinones present
	Combined anthraquinone test	Pink red color precipitate	Anthraquinone derivative present
Cardiac Glycosides	Legal test	Deep red color that fades to brownish yellow	Cardenolides present
	Keddes test	Violet color that fades to brownish yellow	Lactone ring of Cardenolides present
	Lieberman's test	Violet color precipitate	Steroidal nucleus present
	Keller-Kiliani test	Brownish ring at the interface and violet ring below.	Steroid ring of Glycosides present.
Terpenoids	Salkowski test	Light turbid red brown color	Terpenoids present
Flavonoids	Ferric chloride test	Woolly light brown precipitate	Phenolic nucleus present
	Lead acetate test	Dirty brownish precipitate	Flavonoids present
	Sodium hydroxide test	Golden yellow precipitate	Flavonoids present
Tannins	1ml Ferric Chloride + Extract	Blue green precipitate	Tannin present
Phlobatannins	1% HCl + Extract	Reddish precipitate	Phlobatannins present
Cyanogenic Glycosides	Sodium picrate + Extract heated for 5 minutes	No brownish color found	Cyanosides absent
Reducing Sugar Compounds	Fehling test	Deep blue-green color appears	Hexose sugar present
	Barfoed test	Red precipitate	Monosaccharides present
	Resorcinol test	Deep yellow precipitate	Keto sugar confirmed
	Phloroglucinol test	Reddish yellow precipitate	Pento sugar present

### 3.7 Effects of DAE and ALX on Testicular Histomorphometrics (Stereology)

Evaluating and interpreting histological sections with stereological principles helps to unravel some of the essential issues with qualitative microscopic investigation and helps provide answers to important questions about the spermatogenic process.

As shown in Table 5, the mean seminiferous tubular diameters of the diabetic untreated rats were significantly reduced ( $104.1 \pm 4.3 \mu\text{m}$ ) as compared to that of the control groups ( $173.3 \pm 6.2 \mu\text{m}$ ). However, there was a significant increase ( $P < 0.05$ ) in the tubular diameter of group treated with extract of 100 mg/kg and 300 mg/kg DAE ( $139.2 \pm 5.2 \mu\text{m}$  and  $151.2 \pm 4.1 \mu\text{m}$ ) as compared to tubular diameter of the control groups. The disparity in the cross-sectional area

**Table 3. Effects of oral administration of *D. arborea* extract after 8 weeks on hematological parameters in ALX-diabetic male rats**

Hematological parameters	Group			
	A	B	C	D
RBC×10 <sup>6</sup>	7.1±0.6	4.2±0.1 <sup>a</sup>	6.1±0.03 <sup>b</sup>	6.5±0.05 <sup>b</sup>
Hb (g/dl)	13.3±0.8	7.7±0.5 <sup>a</sup>	10.5±1.6 <sup>b</sup>	12.6±1.5 <sup>b</sup>
PCV (%)	20.0±2.7	15.6±0.4	18.9±1.6 <sup>b</sup>	26.0±2.2 <sup>a,b</sup>
WBC×10 <sup>3</sup>	4.3±0.1	4.1±1.0	5.5±0.5 <sup>a,b</sup>	6.3±0.2 <sup>a,b</sup>
MCV(FL)	57.4±3.0	51.1±2.5	52.4±1.6	52.9±3.0
MCH(pg)	18.8±1.5	18.2±1.2	18.3±0.05	18.9±2.1
MCHC(g/dl)	32.8±3.5	35.6±3.5	31.2±1.02	32.4±0.7

Values are the mean ± SEM for 6 rats in each group; a: Statistically significant when compared to control group (A) at  $P < 0.05$ ; b: Statistically significant when compared to diabetic untreated group (B) at  $P < 0.05$ . MCH: mean cell hemoglobin; MCHC: mean cell hemoglobin concentration, and MCV: mean cell volume

**Table 4. Effects of oral administration of with *D. arborea* extract after 8 weeks on testicular weights, sperm count and sperm motility in ALX-diabetic male rats**

Spermiogram parameter	Group			
	A	B	C	D
Testis weight (g)	1.493±0.241	0.877±0.186 <sup>a</sup>	1.203±0.103 <sup>b</sup>	1.442±0.134 <sup>b</sup>
Epididymis weight (g)	0.677±0.184	0.265±0.052 <sup>a</sup>	0.582± 0.155 <sup>b</sup>	0.643±-0.125 <sup>b</sup>
Sperm count x 10 <sup>6</sup> (ml)	26.5±0.052	16.40±4.700 <sup>a</sup>	25.20±6.700 <sup>b</sup>	29.20±3.600 <sup>b</sup>
Sperm motility (%)	87.90±9.400	26.70±10.700 <sup>a</sup>	68.40±7.500 <sup>b</sup>	79.60±8.700 <sup>b</sup>
Normal sperm morphology (%)	95.30±2.100	36.5±6.1 <sup>a</sup>	77.2±0.1 <sup>b</sup>	89.4±0.4 <sup>b</sup>
Abnormal sperm morphology (%)	4.7±5.400	63.5±3.2 <sup>a</sup>	22.8±2.1 <sup>b</sup>	10.6±1.1 <sup>b</sup>

Values are the mean ± SEM for 6 rats in each group; a: Statistically significant when compared to control group (A) at  $P < 0.05$ ; b: Statistically significant when compared to diabetic untreated group (B) at  $P < 0.05$

of the seminiferous tubules, the number of tubular profiles per unit area and the mean numerical density of seminiferous tubules of both the diabetic treated (C & D) and untreated (B) followed a similar pattern as the tubular diameter (C: 15.3±3.9, 13.5±1.4, 6.9±2.8; D: 20.3±1.1, 19.1±4.1, 6.2±8.1 and A: 25.1±3.6, 25.4±8.1, 7.8±1.6) respectively.

### 3.8 Effects of DAE and ALX on Testicular Histopathological Profiles

Testicular tissue sections of the control rats (group A) showed normal testicular cytoarchitecture with distinct seminiferous tubules composed of both sertoli and spermatogenic cells. Majority of the spermatogenic cells were seen to be at the spermiogenic stage of differentiation. Interstitial or leydig cells were also prominently interspersed between the seminiferous tubules (Fig. 5A). The testes of group D treated with DAE had a more stable and well-arranged seminiferous tubules compared to group C and B. Vacuoles were observed due to recovery, but were not as numerous as those observed in the diabetic

control group (B). Dispersed spermatogenic and sertoli cells were also found in the testes of these rats (Fig. 5B). Testicular histology of the diabetic control groups (B) showed alteration and distortion of both germinal epithelia and seminiferous tubules (Fig. 5B). The peritubular tissue surrounding the seminiferous tubules and interstitial cells were altered. There was presence of large vacuoles within the seminiferous tubules (Fig. 5B). The testicular cytoarchitecture of rats in group C treated with 100 mg/kg DAE showed a better improvement; distinct seminiferous tubules were observed (Fig. 5C). Numerous boundary cells as well as sertoli cells were found in the epithelial lining of the tubules. Remarkably, appreciable number of blood vessels was observed in the connective tissue of the seminiferous tubules (Fig. 5C). The testes of group D rats treated with 300 mg/kg DAE showed a significant improvement in the spermatogenic arrangement (Fig. 5D). There was an increase in the leydig cells; well-defined seminiferous tubules were also observed. The lumens of seminiferous tubules were filled with active sperm cells.

### 3.9 Effects of DAE and ALX on Pancreatic Histopathological Profiles

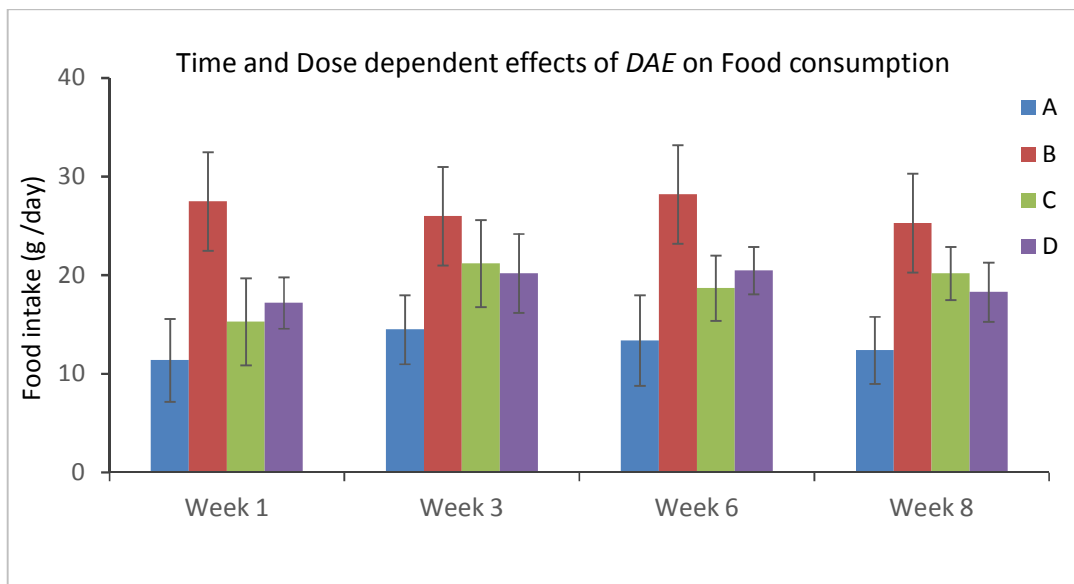
On the other hand, the histological sections of the pancreatic parenchyma showed significant differences in the population of  $\alpha$ - and  $\beta$ - cells of the pancreatic islet. Since the pancreatic  $\beta$ - cells

are involved in the synthesis of insulin, the Alloxan monohydrate was found to cause the massive destruction and progressive loss of  $\beta$ -cells mass as well as degeneration of islets of in the pancreatic tissue sections examined (Fig. 6B). Thereby, disrupting the metabolism of glucose due to the insulin deficiency.

**Table 5. Effects of ALX and DAE on seminiferous tubular diameter ( $\mu\text{m}$ ), cross sectional area ( $\mu\text{m}^2$ ), numerical densities of seminiferous tubules ( $\mu\text{m}^{-3}$ ) and number of profiles per unit area ( $\mu\text{m}^{-2}$ ) (Testicular histomorphometrics) of sprague dawley rats**

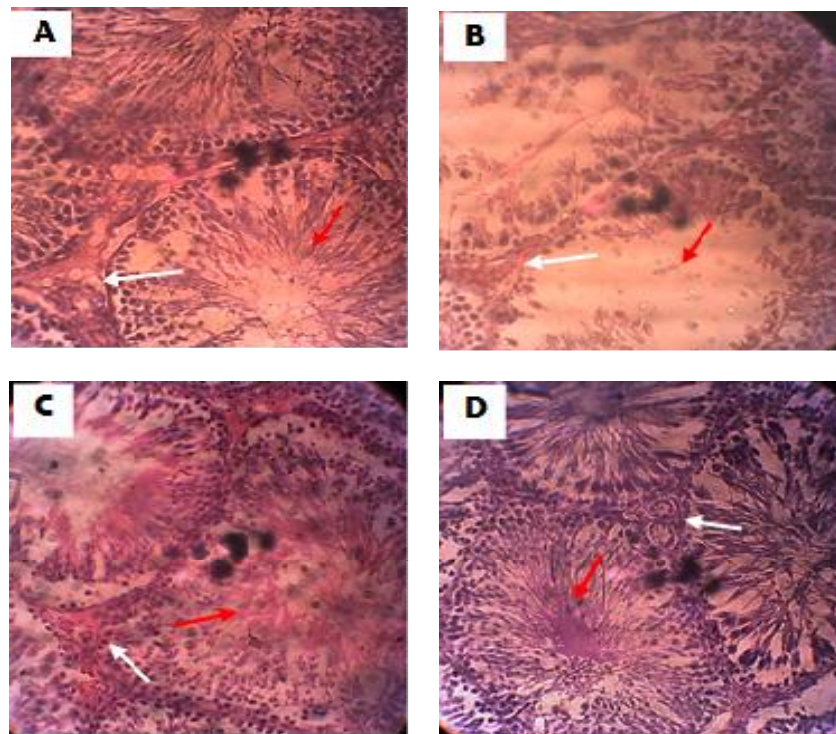
Group	Testicular histomorphometric parameters			
	D( $\mu\text{m}$ )	A <sub>c</sub> ( $\times 10^3 \mu\text{m}^2$ )	N <sub>A</sub> ( $\times 10^{-8} \mu\text{m}^{-2}$ )	N <sub>v</sub> ( $\times 10^{-10} \mu\text{m}^{-3}$ )
A	173.3 $\pm$ 6.2	32.4 $\pm$ 2.8	36.0 $\pm$ 7.1	11.2 $\pm$ 0.3
B	104.1 $\pm$ 4.3	15.3 $\pm$ 3.9 <sup>a</sup>	13.5 $\pm$ 1.4 <sup>a</sup>	6.9 $\pm$ 2.8 <sup>a</sup>
C	139.2 $\pm$ 5.2	20.3 $\pm$ 1.1 <sup>a, b</sup>	19.1 $\pm$ 4.1 <sup>a, b</sup>	6.2 $\pm$ 8.1 <sup>a, b</sup>
D	151.2 $\pm$ 4.1	25.1 $\pm$ 3.6 <sup>a, b</sup>	25.4 $\pm$ 8.1 <sup>a, b</sup>	7.8 $\pm$ 1.6 <sup>a, b</sup>

Values are the mean  $\pm$  SEM for 6 rats in each group; a: Statistically significant when compared to control group (A) at  $P < 0.05$ ; b: Statistically significant when compared to untreated diabetic group (B) at  $P < 0.05$ . D = Tubular diameter, A<sub>c</sub> = Cross sectional area, N<sub>A</sub> = Numerical densities of seminiferous tubules, N<sub>v</sub> = Number of profiles per unit area



**Fig. 4. Effects of oral administration of *D. arborea* extract for 8 weeks on food intake (g/day) in ALX-diabetic male rats**

Graph showing the time and dose dependent effect of DAE treatments on food consumption (appetite) following ALX-induced diabetic condition in rats of between group A and B, and group C and D. Rats were grouped into 4 comprising of normal control (A), diabetic control (B), diabetic rats treated with 100 mg/kg of DAE and lastly diabetic rats treated with 300 mg/kg of DAE. Values are represented as the mean  $\pm$  SEM for the 6 rats per group. a: Statistically significant when compared to control group (A) at  $P < 0.05$ ; b: Statistically significant when compared to diabetic untreated group (B) at  $P < 0.05$



**Fig. 5. Micrographs of the histopathological slides of testicular sections (H&E) of group A-D rats following exposure to ALX and DAE**

A: Showing the micrograph of normal histological section (x 400) of group A (treated with 0.5 ml/kg body weight of acacia solution for 8 weeks), with active maturing seminiferous tubules (epithelium) associated with normal spermatogenic series and sperm cells. B: Showing the micrograph of histological section (x 400) of group B (diabetic control group; treated with Alloxan monohydrate alone for 8 weeks), with marked degeneration of most seminiferous tubules and expansion of the interstitial spaces between each seminiferous tubules. C: Showing the micrograph for histological section (x 400) of group C (diabetic group; treated with DAE (100 mg/kg following initial exposure to ALX) with mild degeneration of some seminiferous tubules with partial recovery and normal spermatogenic series in the most seminiferous tubules. D: Showing the micrograph for histological section (x 400) of group D (diabetic group; treated with DAE (300 mg/kg) following initial exposure to ALX) with normal histological structure of most seminiferous tubules and presence of sperms in the lumen of most seminiferous tubules. Note: Dark spots found on each histomicrograph are microscopic artifacts)

#### 4. DISCUSSION

Diabetes mellitus is a chronic metabolic disease with debilitating complications. Two types are mostly described in literatures; type-1 (Insulin dependent diabetes mellitus) and type-2 (Insulin resistant diabetes mellitus) [27]. These two interestingly, though have distinct pathogenesis, share similar life threatening complications such as long-term hyperglycemia, which is associated with many other complications, including male reproductive dysfunctions and infertility in diabetic patients [5]. Over 90% of diabetic patients are known to suffer from severe insulin resistance, which leads to severe metabolic and reproductive complications [9,10].

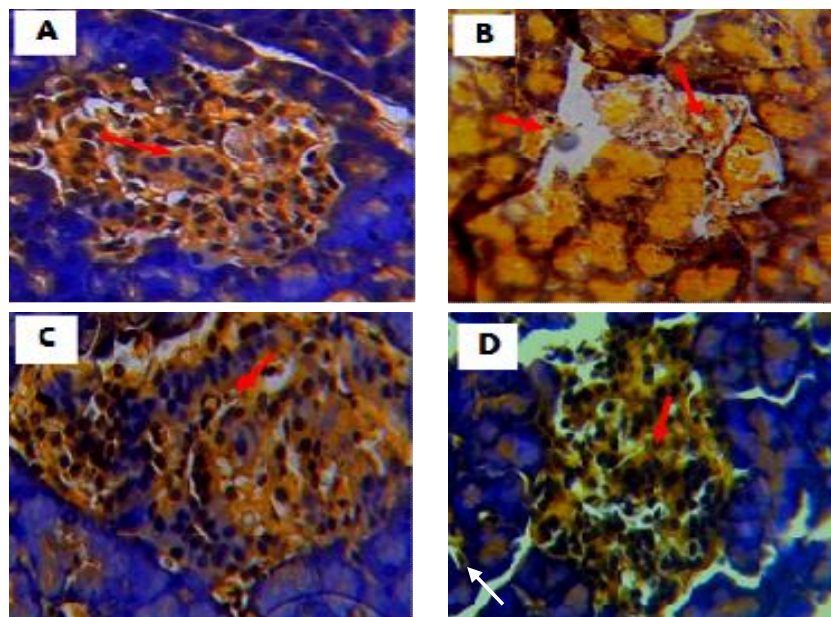
The aim of the present study is to investigate the phytotherapeutic effects of ethanolic extract of

*Dracaena arborea* on the blood glucose level, appetite, spermiogram, and testicular and pancreatic cytoarchitecture (histomorphometrics and/or histopathology) in type-1 Alloxan-induced diabetic Sprague dawley male rats. In this study, the administration of Alloxan monohydrate (ALX) caused the massive quantitative destruction and loss of the  $\beta$ -cells of the pancreatic islet while sparing the  $\alpha$ -cells. Beta-cells ( $\beta$ -cells) are known to be relatively more susceptible to ROS injury by ALX as they have lower activity of antioxidant enzymes superoxide dismutase, catalase and glutathione peroxidase [28]. This is accompanied with lower activity of an important endogenous antioxidant thioredoxin. It's been demonstrated that initially the activity of antioxidant enzyme production increases to counter the increased ALX activity, but beta-cells have limited capacity

to adapt [29]. Usually when  $\beta$ -cells become defective or die as observed, the alpha cells within the pancreas become accustomed to lower levels of  $\beta$ -cells or non-functioning  $\beta$ -cells and thus lose their ability to sense the level of glucose in the bloodstream appropriately [30,31]. This confuses both the  $\alpha$ -cells and the liver, while an inadequate presence of appropriately functioning  $\beta$ -cells in the pancreas will cause insulin deficiency (in which insulin secretion will not be enough to convert the excess glucose in the blood stream to glycogen for storage in the liver and muscles) [32]. The  $\alpha$ -cells are put in the state of dilemma in which they won't know when to secrete glucagon and when not to, thus resulting in over secretion of glucagon rather than under secretion of glucagon. This leads to the elevation of glucose levels and eventually an insulin deficient diabetes and complication [33].

Insulin is well known as an anabolic hormone that plays a vital role in maintenance of body

growth and overall body metabolism. Partial or complete insulin deficiency in diabetic humans as well as in induced diabetic experimental animals appears to have adverse effects on all organs, including reproductive organs [33]. Since male fertility is dependent on the health of the testis and the gonadal endocrine system. When the testis is in good physiological state, and functioning normally, it allows for normal spermatogenesis, which favors normal production of mature as well as viable sperm cells [34]. Though, normal reproductive function is regulated by the hypothalamic-pituitary-gonadal axis, studies have shown that certain local gonadal factors such as those of the insulin/IGF (insulin-like growth factors I (IGF-1) and II (IGF-2)) family may modulate reproductive performance [35]. Hence, a deficiency in insulin secretion may affect the functions of these factors in providing the essential signals for the development of the testis, as well as the control of metabolism and reproductive functions in diabetic patients [35,36].



**Fig. 6. Micrographs of the histopathological pancreatic tissue sections (Aldehyde Fuchsin) of the group A- D rats following exposure to ALX and DAE**

*A: Showing the normal histological section (x 400) of group A (treated with 0.5 ml/kg body weight acacia solution for 8 weeks), with normal morphology of pancreatic islet with dense presence of darkly stained  $\beta$ -cells. B: Showing the histological section (x 1000) of group B (diabetic control group; treated with Alloxan monohydrate only for 8 weeks), with showing severe necrosis of  $\beta$ -cells. C: Showing the histological section (x 400) of group C (diabetic group; treated with DAE (100 mg/kg following initial exposure to ALX) with a mild areas of necrosis and spots of surviving  $\beta$ -cells widely dispersed. D: Showing the histological section (x 400) of group D (diabetic group; treated with DAE (300 mg/kg) following initial exposure to ALX) with mild necrotic changes with cluster of surviving  $\beta$ -cells. Red arrow: pointing to population of  $\beta$ -cells in the pancreatic islet of Langerhans in slide A-D*

Numerous studies in the past have demonstrated and validated the ability of ALX to induce type-1 diabetes mellitus in different animal models [4,21,22,30,37,37]. Sprague dawley rats were used for this experiment, since studies have shown that they are one of the favorite wild-type strains for studies on diabetes mellitus [28]. The metabolic abnormalities and responses exhibited by these animal models due to ALX-induced toxicity are similar to those seen in diabetic patients. These rats were also found to have high fasting and non-fasting blood glucose levels similar to diabetic individuals [12,30].

The blood glucose status has been used clinically as a biologic dosimeter to determine response to therapeutic intervention for diabetes mellitus. Sustained reduction in hyperglycemia plays a significant role in the prevention or reversal of diabetic complications and improving the quality of life of diabetic patients [9]. It also decreases the risk of developing microvascular complications and other reproductive dysfunctions [38]. A significant time- and dose-dependent improvement ( $P < 0.05$ ) in the blood-glucose status for DAE treated rats were observed during this study.

Apart from the symptoms such as lethargy, fatigue, weight loss, and fever observed in diabetic patient, loss of appetite is another common occurrence. In such patient, leptin, a hormone that plays an important role in appetite control is usually produced in high levels resulting in high ROS generation or oxidative stress in diabetic patients [39]. The major determinant of leptin secretion has been shown to be glucose metabolism [35]. The results also indicated that water and food intake (appetite) increased significantly ( $P < 0.05$ ) after administration of ALX in male rats. These findings are consistent with previous studies which indicated that the cytotoxic action of ALX is mediated by the formation of free radicals such as superoxide and hydroxyl radicals [39].

Appreciable normalization ( $P < 0.05$ ) of RBC, PCV and Hb levels were also observed in the groups of diabetic rats treated with the 100 mg/kg and 300 mg/kg DAE. There was also a slight rise in the mean leukocyte counts of the treated groups, which could have been in defense to toxic environment [40]. The observed increase in the hemoglobin level in all the treated groups could be due to the increase in iron absorption. There was no significant difference ( $P > 0.05$ ) in MCHC for the treated rats

compared to the control. However, increase in MCHC value has been associated with anemic conditions [40]. No statistically significant difference ( $P > 0.05$ ) in MCV was found in all groups signifying that the extracts did not cause regenerative anemia.

Studies have shown that diabetic conditions could alter one or more stages of spermatogenesis, thereby causing oligospermia r aspermia in such individuals [41]. In this study, ALX administration was found to cause severe testicular derangement that led to the dramatic change in the testicular morphology and altered spermatogenic process or spermiogram in the rats. A significant increase ( $P < 0.05$ ) in the population of abnormally shaped sperm cells were also observed compared to the normal controls as well as the DAE treated rats. These effects appeared to be mediated through the oxidative stress and excess ROS generated [42,43] due to hyperglycemia and insulin deficiency as observed in one of our study on roles DAE plays in ameliorating the testicular oxidative stress-mediated disorders in diabetic rats [29]. These excess in free radicals generated during diabetic conditions have been demonstrated to be one of the major causes of genomic instability or mutagenicity such as chromosome aberrations, DNA fragmentation, micronuclei and sperm abnormalities [44,45]. It will then be not be unwise to hypothesize that the mechanisms by which testicular damages and sperm abnormalities are induced in diabetic conditions may be due to the activation of several damaging pathways by ROS, such as the accelerated formation of advanced glycation end production, polyol pathway, hexosamine pathway, protein kinase or increase of lipid peroxidation [46].

In this study, cytoarchitectural (histomorphometry and histopathology) examination of the testes of diabetic rats revealed changes in testicular structural architecture such as marked degeneration of many of the seminiferous tubules in the testicular tissues. Other lesions observed include atrophied seminiferous tubules. Spermatogenic derangement in the diabetic group as observed in the histomicrographs revealed areas of atrophied seminiferous tubules with absence of spermatogenic series and sperm cells in the tubular lumen. The testicular histomorphometry revealed a significant decrease ( $P < 0.05$ ) in the diameter of the seminiferous tubules as well as the height of germinal epithelium of the testis and epididymis

compared to those in the normal healthy controls. This is characterized by the decrease in the diameter of the seminiferous tubules and decrease in the thickness of the basement membrane of the seminiferous tubule. Reduction in the spermatogenic cell series was also observed as well as modifications in morphology of sperm cells, disorganization and degeneration of spermatocytes, spermatids and germ cells. The atrophy of the testes may be due to the decrease in testosterone level associated with the absence or diminution of serum insulin levels, since insulin acts as an anti-apoptotic factor capable of regulating testicular apoptosis and sexual dysfunction induced by diabetes [20-24].

Atrophy of the seminiferous tubule and decrease in spermatogenic cells are important indicators of spermatogenesis failure. Diabetes also increases the thickening of the seminiferous tubules' basement membrane. This thickening is accompanied by a decreased rate of sperm production and an overall reduction in the size or diameter of seminiferous tubules [25,37,38]. These changes may be due to ALX- induced diabetic conditions, which cause delicately complex molecular changes in the testicular signaling pathway, which are very important in maintaining normal spermiogram, thereby altering the conventional sperm parameters.

Oral administration of *Dracaena arborea* leaf extracts for a period of eight weeks for the first time was found to improve spermiogram (sperm motility and sperm count), appetite (food or water intakes) and glucose metabolism. Recent evidence already revealed that extracts of various parts of *Dracaena arborea* plant may exhibit powerful antioxidant activity against various oxidative systems *in vivo* [47]. The antioxidant activity of these extracts may be attributed to the reduction or amelioration of oxidative-stress induced diabetic complications such as lipid peroxidation, by elevation of antioxidant enzyme activities as observed from our prior study [29]. In addition, previous studies involving treatments with antioxidant compounds derived from natural plant products already demonstrate their importance in regulating  $\beta$ -pancreatic cell functions and growth, thereby reducing the complications due to diabetes [30,39].

The modulating effects of *DAE* may perhaps also be linked to the activities of other bioactive compounds in the extract that may have anti-diabetic and/or insulinomimetic activity resulting

in glucose tolerance, increase in glucose utilization and metabolism in peripheral tissues [48]. Aligning our results with previous studies that used other antioxidant medicinal plants, we can indicate that leaf extracts of *Dracaena arborea* contain bioactive compounds with antioxidant activities that can ameliorate the toxic and diabetic effect of ALX on pancreatic and testicular cells [29,38,40]. Some of these phytochemical compounds present in *DAE* may also be responsible for the restoration of  $\beta$ -cells' integrity and metabolic functions, while at the same time ensuring maximum synthesis of insulin by these cells necessary for glucose tolerance [30]. This may also help to reverse the sexual or reproductive dysfunctions associated with diabetes in men [47].

This study further reinforced previous findings that intraperitoneal administration of high doses of ALX in male rats induce type-1 diabetogenic conditions, which leads to reproductive complications such as reduced testicular and epididymal weights (shrinkage), decreased testosterone production, reduced sperm motility and sperm counts, and also decrease in the gonadal function of both leydig (testosterone producing) cells and sertoli (supporting) cells [26,33]. Invariably, this suggests that ALX negates the cytoarchitectural and functional integrity of testicular tissues via a mechanism which may involve insulin deficiency [33,35].

For a better understanding of the molecular mechanisms by which *DAE* normalize blood glucose levels, ROS levels, and sexual disorders as well as ameliorate pancreatic and testicular dysfunctions in diabetic patients, more in depth studies are needed, especially to investigate the role each screened phytochemical constituents plays in the anti-diabetic and fertility effects of *DAE*.

## 5. CONCLUSIONS

In conclusion, the present study has demonstrated that administration of ALX in rats induces hyperglycemia and exhibits a number of defects in reproductive organs of male rats. On the other hand, ethanolic leaf extract of *Dracaena arborea* exhibits anti-diabetic, anti-hyperglycemic, antioxidant and reduced sperm abnormalities as well as increased spermiogram in ALX-induced diabetic male rats. This finding is in agreement with prior studies involving the use of antioxidant medicinal plant extracts in the treatment of diabetic conditions and

complications. This study has further reiterated the promising benefits of medicinal *D. arborea* extracts as a phytotherapeutic herbal drug that could prevent the development of diabetic complications and improve the potency or reproductive performance of male animals and humans.

## CONSENT

It is not applicable.

## ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee of our Institution". All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 "Declaration of Helsinki".

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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