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Dracaena arborea leaf extract: A phytotherapeutic option for ameliorating oxidative stress-mediated endocrine and testicular disorders in alloxan-induced diabetic rats

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ABSTRACT

Objective: To explore and exploit these anti-diabetic properties of the leaf extracts of *Dracaena arborea* by investigating its therapeutic effects on blood glucose regulation, testicular and endocrine disorders in alloxan (ALX)-induced type-1 diabetic rats.

Methods: Rats were divided into four groups of five animals each in a completely randomized trial and treated. ALX and then *Dracaena arborea* extracts (DAE) at 100 mg/kg and 300 mg/kg/day were administered to both normal (Group A) and diabetic rats (Groups C and D) and none to the diabetic control group (Group B) for a period of 8 weeks. The serum level of glucose, follicle stimulating hormone, luteinizing hormone and free testosterone were estimated for all treated and control groups. Oxidative stress parameters such as superoxide dismutase, glutathione peroxidase, glutathione reductase, catalase, and malondialdehyde were also estimated from testicular cells of control, untreated diabetic and DAE-treated diabetic rats.

Results: We observed a significant ($P < 0.05$) decrease in the serum glucose level as well as a drug-induced dose-dependent reduction in testosterone, follicle stimulating hormone and luteinizing hormone in the rats following type-1 diabetic conditions induction by ALX. There was a significant dose dependent response ($P < 0.05$) to treatment between the DAE-treated diabetic groups. Furthermore, a significant decrease ($P < 0.05$) in oxidative stress parameters was observed in testicular cells of diabetic rats post-treatment.

Conclusions: These findings suggest that DAE is a potential beneficial phytotherapeutic agent that may be used in ameliorating hyperglycemic, endocrine and reproductive disorders in type-1 diabetic men.

1. Introduction

Diabetes mellitus is a chronic, degenerative and metabolic disorder of public health significance, characterized by hyperglycemia resulting from defective insulin secretion that could lead to many complications[1]. Unfortunately, statistical evidence according to data from the International Diabetic Federation revealed a continual increase in the prevalence of this disease. In 2011, over 366 million people worldwide were diagnosed with diabetes, and this is expected to rise to 522 million by 2030[2].

There is growing evidence from published literatures that

increased oxidative stress in diabetes mellitus patients may be due to overproduction of reactive oxygen species (ROS), which may lead to a decrease in the efficiency of the antioxidant defense mechanisms[3]. The increased oxidative stress was shown to occur very early in diabetics and noted to worsen the disease overtime[3-5].

Oxidative stress generated by hyperglycemia, hyperlipidemia or defects in the antioxidant defense mechanisms has been demonstrated to be an important mediator of diabetic complications[3,6,7]. Sexual dysfunction and impairment of fertility are well recognized diabetic complications among male diabetes mellitus patients and also in experimental animals during *in vivo* studies[4,5]. Other complications observed include severe destruction of the β -cells of the pancreas leading to insulin deficiencies and severe disturbances of carbohydrate, fat, and protein metabolism[6]. Since the imbalance between the production of ROS and antioxidant

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enzyme activities leads to oxidative stress in tissues and cells[7], ROS has been implicated in the pathology of several human diseases, such as atherosclerosis, inflammation, cancer, rheumatoid arthritis, and neurodegenerative diseases like Alzheimer's disease and multiple sclerosis[8].

Studies in both human and animal models have shown that insulin-dependent diabetes mellitus (type 1) often manifest lots of reproductive complications such as low testosterone secretion, abnormal spermiogram, decrease in testicular weight, and ultrastructural changes in testicular architecture[5,8]. Other complications reported include loss of spermatid germ cells by apoptotic cell death, impotency, retrograde ejaculation, hypogonadism and loss of libido[8].

Meanwhile, medicinal plants with antioxidant properties have attracted a great deal of research in recent times due to their beneficial effects and ability to ameliorate some of these medical conditions and symptoms[9]. Recent studies have been focused on the identification of antioxidants of natural origin with powerful free radical scavenging capabilities[7]. One of such promising plant with antioxidant properties is *Dracaena arborea* (*D. arborea*), which belongs to the family Asparagaceae[10]. The genus is composed of about 500 species of woody stemmed plants mostly native to countries in the tropics and subtropics[10,11]. *D. arborea* root and leaf extracts (DAE) have since been used locally in many countries as aphrodisiac to treat sexual inadequacy and to stimulate sexual vigor[11,12].

Alloxan (ALX) monohydrate is a strong oxidizing agent that has diabetogenic properties due to its ability to destroy β -cells of the pancreatic islet, possibly by excess free radical generation leading to oxidative stress conditions[11]. ALX monohydrate and its reduction product; dialuric acid, generates a futile redox cycle that leads to the formation of superoxide radicals[12]. These radicals undergo dismutation to hydrogen peroxide leading to the formation of highly reactive hydroxyl radicals via Fenton reaction. The action and reaction of these ROS with a simultaneous massive increase in cytosolic calcium concentration result in the rapid destruction of β -cells in the pancreas and hence other diabetic complications[12].

Understanding the mechanism by which type-1-diabetes negates spermatogenesis, steroidogenesis and other reproductive functions will be a landmark in the development of therapeutic interventions that will help to ameliorate these complications in male diabetic patients[13]. The aim of this study is to investigate the use of DAE as a phytotherapeutic option in reducing oxidative stress mediated testicular and endocrine disorders in ALX-induced type-1 diabetic rats.

2. Materials and methods

2.1. Plant collection

D. arborea tree branches were harvested from a deciduous forest in Ogbomoso, 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 extracts

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 g 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 h. 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[13].

2.3. Experimental design

Male Wistar albino rats weighing between 160 and 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-h light: dark cycles, 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 2 weeks prior to start of experiment. This 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[14] 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[15].

2.4. Induction of type-1 diabetic condition

Diabetic condition was induced experimentally in rats fasted 12 h prior to injection with a single dose of 100 mg/kg ALX monohydrate (Sigma, St. Louis, MO, USA) dissolved in normal saline and administered intraperitoneally[8]. Two hours after the administration of ALX, all rats were given an intraperitoneal injection of glucose 5% in order to overcome ALX-induced hypoglycemia due to the destruction of the pancreatic β -cells and massive release of insulin. Fasting blood glucose level (diabetic status) was measured 48 h 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 150 mg/dL were considered diabetic.

2.5. Animal grouping

One week after the confirmation of the diabetic state by ALX, rats were divided into four groups of five animals each in a completely randomized trial and treated as follows;

Group A: Healthy normal rats given 0.5 mL/kg body weight per day of peanut oil (healthy control group).

Group B: Diabetic rats given 0.5 mL/kg body weight per day of peanut oil (diabetic control group).

Group C: Diabetic rats treated with 100 mg/kg body weight per day of *D. arborea* leaf extract.

Group D: Diabetic rats treated with 300 mg/kg of body weight per day of *D. arborea* leaf extract.

The vehicle (peanut oil) 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 EC₅₀ (concentration causing 50% of maximum effect for any measured biological effect of interest) at 300 mg/kg of DAE from our preliminary experiments was used as therapeutic dose. Initial and final weights as well as the weekly changes in blood glucose measurements were also estimated in this study.

2.6. Serum parameters

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 gonadal hormone titer determination. Follicle stimulating hormone (FSH), luteinizing hormone (LH) and free testosterone (T_F) levels in serum were also estimated by tube-based enzyme immunoassay (EIA) kit (DiaSys, Germany) and the absorbance (optical density) read at 450 nm as described by Raji *et al.*[16].

2.7. Testicular tissues preparation

Eight weeks post treatment, rats were sacrificed under chloroform anesthesia. The testes 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. About 1 g of each organ was cut into small pieces homogenized with an Ultra Turrax homogenizer in 2 mL ice-cold (4 °C) appropriate buffer (pH 7.4) and centrifuged at 1800 r/min for 15 min. The pellet was discarded and a portion of supernatant was again centrifuged at 3200 r/min for 20 min to obtain post-mitochondrial supernatant, which was aliquoted and stored at -60 °C.

2.8. Determination of testicular enzymatic antioxidants

2.8.1. Estimation of DAE and ALX-induced testicular catalase (CAT) activity

CAT activity was estimated based on the method of Aebi[17]. About 0.1 mL of the testicular homogenate (supernatant) was pipetted into cuvette containing 1.9 mL of 50 mmol/L phosphate buffer, pH 7.0. Reaction was started by the addition of 1.0 mL of freshly prepared 30% (v/v) hydrogen peroxide (H₂O₂). The rate of decomposition of H₂O₂ was measured spectrophotometrically observing changes in absorbance at 240 nm. Activity of enzyme was expressed as units/mg protein.

2.8.2. Estimation of DAE and ALX-induced testicular superoxide dismutase (SOD) activity

SOD activity was studied according to the method described by Winterbourn *et al.*[18]. The principle of this assay was based on the ability of SOD to inhibit the reduction of Nitro-blue tetrazolium

(NBT). The reaction mixture contained 2.7 mL of 0.067 mol/L phosphate buffer, pH 7.8, 0.05 mL of 0.12 mmol/L riboflavin, 0.1 mL of 1.5 mmol/L NBT, 0.05 mL of 0.01 mol/L methionine and 0.1 mL of enzyme samples. Uniform illumination of the tubes was ensured by placing it in air-tight aluminum foil in a box with a 15W fluorescent lamp for 10 min. Controls without the enzyme source were also included. The absorbance was measured at 560 nm. One unit of SOD was defined as the amount of enzyme required to inhibit the reduction of NBT by 50% under the specific conditions, expressed in μ/mg protein.

2.8.3. Estimation of DAE and ALX-induced testicular glutathione peroxidase (GPx) activity

GPx activity was evaluated by the method described by Rukmini *et al.*[19]. The reaction mixture contained 2.0 mL of 0.4 mol/L Tris-HCL buffer, pH 7.0, and 0.01 mL of 10 mmol/L sodium azide, 0.2 mL of enzyme, 0.2 mL of 10 mmol/L glutathione and 0.5 mL of 0.2 mmol/L H₂O₂. The contents were incubated at 37 °C for 10 min followed by the termination of the reaction by the addition of 0.4 mL 10% (v/v) trichloroacetic acid (TCA), centrifuged at 5000 r/min for 5 min. The absorbance of the product was read at 430 nm and expressed as nmol/mg protein.

2.9. Determination of testicular non-enzymatic antioxidants

2.9.1. Estimation of DAE and ALX-induced testicular glutathione reductase (GSH) activity

GSH was determined by the method of Sushruta *et al.*[20]. About 1.0 mL of supernatant was treated with 0.5 mL of Ellman's reagent (19.8 mg of 5, 5'-dithiobisnitro benzoic acid in 100 mL of 0.1% sodium nitrate) and 3.0 mL of phosphate buffer (0.2 mol/L, pH 8.0). About 0.4 mL of distilled water was added. The mixture was thoroughly mixed and the absorbance was read at 412 nm, expressed in nmol/mg protein.

2.9.2. Estimation of DAE and ALX-induced testicular malondialdehyde (MDA) activity

Lipid peroxidation in the testicular tissues was studied colorimetrically via thiobarbituric acid reactive substances method as demonstrated by Agbor *et al.*[21]. MDA, a product of lipid peroxidation is the principal component of thiobarbituric acid reactive substances.

Briefly, 0.1 mL of tissue homogenate (Tris-HCL buffer, pH 7.5) was treated with 2 mL of (1:1:1 ratio) TBA-TCA-HCL reagents (thiobarbituric acid 0.37%, 0.25 mol/L HCL and 15% TCA) and placed in water bath for 15 min and cooled. The absorbance was measured against reference blank at 535 nm. Concentration was calculated using the molar absorptivity of MDA which is 1.56×10^5 mol⁻¹·cm⁻¹ and expressed as nmol/mg protein.

2.10. Statistical analysis

All data were expressed as means ± SD. Data were also statistically analyzed using One-way ANOVA followed by student's *t*-test

analysis to test the significance 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 (v. 5) was used to analyze the data.

3. Results

3.1. Effects of DAE on body weight of rats

To determine the effect of treatment with *D. arborea* leaf extract on the body weight of ALX-induced diabetic rats. Weekly body weight of DAE untreated and treated diabetic and control rats were taken. Table 1 shows a significant decrease ($P < 0.05$) in appetite and weight after diabetes induction by ALX. All rats were monitored for gain in body weight. The control group (A) gained weight over the 8 weeks of experimental period, with the mean body weight increasing by 29.9 g after 8 weeks (Table 1). In contrast, the untreated diabetic group (B) lost an average of -25.4 g after 8 weeks ($P < 0.05$). Treatment with *D. arborea* resulted in significant weight gain to levels approaching the control group (Groups C and D, versus Group A). In the untreated diabetic group, weight depreciation occurred throughout the group, while weight appreciation coupled with increase in appetite were observed few days post treatment with DAE in Groups C and D. The negative difference in mean body weight of Group B rats revealed that there was a weight loss due to diabetic condition sequel to exposure to ALX. However a dose dependent gain in mean weight was observed for Groups C and D.

Table 1

Effects of oral administration of ethanolic leaf extract of *D. arborea* for 8 weeks on body weight in ALX-diabetic male rats. g.

Group	Pre-treatment body weight	Post-treatment body weight	Difference in body weight
A	160.4 ± 8.2	190.3 ± 10.8	29.9
B	168.2 ± 1.2	142.8 ± 10.1 ^a	-25.4 ^a
C	173.1 ± 4.3	186.4 ± 7.5 ^b	13.3 ^{ab}
D	176.8 ± 3.1	195.0 ± 8.5 ^b	18.2 ^{ab}

Values are the mean values ± SD, $n = 10$.

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

3.2. Effects of DAE on serum glucose concentration

An increase in serum glucose concentration (mg/dL) was observed in ALX treated rats, most especially in untreated diabetic group after 8 weeks, relative to the control group (Table 2). After 8 weeks, the serum glucose concentration in untreated diabetic group increased to 362.2 mg/dL. In treated diabetic rats (Groups C & D), the serum glucose concentration decreased to 254.2 mg/dL and 242.7 mg/dL respectively, which was significantly lower than ($P < 0.05$) that observed in the untreated diabetic group. A rise in the mean blood glucose level was observed in Group B after treatment with ALX, which worsens with time and was found to be four times higher than the normal average found in Group A. Following treatment with DAE, the initial rise in the blood glucose levels of Groups C

and D rats were ameliorated towards the normal.

Table 2

Effects of oral administration of ethanolic leaf extract of *D. arborea* on serum glucose concentrations in ALX-diabetic male rats for a period of 8 weeks post treatment. mg/dL.

Group	Week 1	Week 3	Week 6	Week 8
A	83.8 ± 4.8	85.2 ± 7.4	91.4 ± 7.8	87.4 ± 4.1
B	343.4 ± 20.2 ^a	349.6 ± 24.4 ^a	357.1 ± 15.8 ^a	362.2 ± 18.9 ^a
C	298.2 ± 21.4 ^b	273.8 ± 23.4 ^b	269.5 ± 25.4 ^b	254.2 ± 20.2 ^b
D	290.4 ± 14.7 ^b	269.3 ± 17.8 ^b	257.8 ± 21.1 ^b	242.7 ± 18.4 ^b

Values are represented as the mean values ± SD, $n = 10$. ^a: Statistically significant when compared to control group (A) at $P < 0.05$; ^b: Statistically significant when compared to diabetic untreated group (B) at $P < 0.05$.

3.3. Effects of DAE on serum levels of testosterone, FSH and LH (gonadal hormones)

The diabetic rats showed a decrease in serum testosterone (T_F), FSH and LH levels compared to control groups (Table 3). Treatment of the diabetic rats with DAE (Groups C & D) caused a significant increase ($P < 0.05$) in the levels of testosterone in a dose-dependent manner. There was a significant difference in the mean testosterone (T_F), FSH and LH levels between Group B rats treated with ALX only compared to Groups A, C and D. DAE was able to cause a significant rise in the endocrine function of the gonads and anterior pituitary gonadotropins (FSH and LH). This suggests that DAE reversed the gonadal endocrine dysfunctional effect of ALX, which is similar what is seen in diabetic patients.

Table 3

Effects of oral administration of DAE on serum testosterone (T_F), FSH and LH levels 8 weeks post treatment in ALX-diabetic male rats.

Group	TF (ng/mL)	FSH (µg/mL)	LH (µg/mL)
A	0.677 ± 0.184	0.14 ± 0.20	0.11 ± 0.01
B	0.387 ± 0.030 ^a	0.10 ± 8.30 ^a	0.06 ± 4.30 ^a
C	0.588 ± 0.048 ^b	0.11 ± 0.30 ^a	0.09 ± 0.01 ^b
D	0.678 ± 0.076 ^b	0.14 ± 0.30 ^b	0.10 ± 0.03 ^b

Values are represented as the mean values ± SD, $n = 10$. ^a: Statistically significant when compared to control group (A) at $P < 0.05$; ^b: Statistically significant when compared to untreated diabetic group (B) at $P < 0.05$.

3.4. Effects on testicular enzymatic and non-enzymatic antioxidants

ALX induction of diabetes resulted in a significant fall ($P < 0.05$) in SOD, CAT and GPx compared to the control. Post treatment with DAE increased significantly ($P < 0.05$) the testicular SOD, CAT and GPx levels in diabetic rats compared with the untreated diabetic group. This rise in SOD, CAT and GPx was found to be dose-dependent. The testicular GSH level in diabetic rats was significantly lower ($P < 0.05$) compared to normal rats. However, the diabetic rats treated (Group D) with the DAE showed a significant increase ($P < 0.05$) in testicular GSH compared to untreated group (C). On the other hand, the level of MDA was significantly higher ($P < 0.05$) in untreated diabetic rats compared to treated and normal rats. Also the level of MDA in diabetic treated groups (Table 4) was significantly lower ($P < 0.05$) compared to untreated diabetic and control groups.

Table 4

Effects of oral administration of DAE 8 weeks post treatment on testicular enzymatic and non-enzymatic antioxidant levels in ALX-diabetic male rats.

Group	CAT ($\mu\text{mol}/\text{mg}$ protein)	SOD (U/mg protein)	GPx (nmol/mg protein)	GSH (nmol/mg protein)	MDA (nmol/mg protein)
A	13.2 \pm 1.4	48.7 \pm 3.0	0.90 \pm 0.20	2.60 \pm 0.20	0.9 \pm 0.1
B	5.2 \pm 2.3 ^a	12.4 \pm 1.3 ^a	0.28 \pm 0.10 ^a	0.89 \pm 0.20 ^a	4.4 \pm 0.4 ^a
C	12.5 \pm 1.9 ^b	38.3 \pm 2.8 ^b	0.65 \pm 0.20 ^b	1.80 \pm 0.10 ^b	1.4 \pm 0.2 ^b
D	12.8 \pm 1.1 ^b	42.5 \pm 2.5 ^b	0.72 \pm 0.30 ^b	2.30 \pm 0.20 ^b	1.2 \pm 0.1 ^b

Values are represented as the mean values \pm SD, $n = 10$. ^a: Statistically significant when compared to control group (A) at $P < 0.05$; ^b: Statistically significant when compared to untreated diabetic group (B) at $P < 0.05$.

4. Discussion

The present study was designed to elucidate the association between diabetic hyperglycemia and diabetes-induced oxidative stress by assessing parameters related to testicular functions in the male Wistar albino rats and eventually investigate the potential of DAE to ameliorate these disorders. Our results revealed that ALX administered intraperitoneally caused a significant alteration in the male reproductive milieu by markedly inhibiting serum testosterone level, causing abnormal spermiogram (data not shown) and excess generation of ROS by testicular cells. Current data also revealed that after administration of ALX to the male rats, the blood glucose level increased significantly, while the body weight of ALX-induced diabetic rats decreased significantly. Oral administration of ethanolic leaf extract of *D. arborea* in diabetic male rats at doses of 100 mg/kg and 300 mg/kg for 56 days concurrently increased the average body weights, decreased the blood glucose level, and increased the serum testosterone, FSH and LH levels.

The cytotoxic action of ALX may be mediated by the formation of free radicals such as superoxide and CAT radicals[22]. ALX administration in animals had been demonstrated to result in an increase in lipid peroxidation, an indication of tissue damage[22]. Since the sperm plasma membrane has a high content of polyunsaturated fatty acids that is easily susceptible to lipid peroxidation caused by oxidative stress; excessive ROS production that exceeds critical levels can overwhelm all antioxidant defense strategies of spermatozoa and seminal plasma causing oxidative stress that damages the biological membranes in the testes and eventually the spermatogenic and leydig cells, which disrupts spermatogenesis and reduces sperm counts[23,24].

Estimation of SOD, CAT, GPx, MDA, as well as GSH levels along with their antioxidant enzyme activities in biological tissue have been used previously as markers for tissue injury and oxidative stress[24].

In this study, the testicular oxidative stress markers were compared between control, untreated-diabetic and treated diabetic groups. Testicular oxidative damage induced by ALX caused a significant decrease in the activities of antioxidant enzymes (SOD, CAT and GPx) and also GSH with a concurrent significant increase ($P < 0.05$) in levels of MDA as compared to the control group. Whereas, when diabetic rats were treated with the DAE, it markedly ameliorated the oxidative stress mediated damage that was induced by ALX in the rats by normalization of the activities of the antioxidant enzymes.

It is therefore, not unwise to suggest that this extract is safe and might confer protection against testicular damage in diabetics, as evidenced by normalization of the antioxidant enzyme activities and

gonadal endocrine function in treated diabetic groups. The extract also caused an increase in the weight of the testes post treatment[25], which was accompanied by an increase in the serum levels of testosterone. Similar findings have been reported with other plant extracts such as *Quassia amara*, *Azadirachta indica*, *Morinda lucida*, *Zingiber officinale* and *Nigella sativa* in rat models[24,26,27].

Interestingly, glucose oxidation and utilization are important means by which spermatozoa derives energy for their motility[28]. Plethora of studies have shown that testicular weight reduction are often accompanied by decrease in serum testosterone levels in male rats given ALX intraperitoneal injections[26,27,29]. Since most organs required for male reproduction have androgen receptors, they may serve as indicators for the leydig cell functions and/or testosterone synthesis, which in association with follicle stimulating hormone acts on the seminiferous tubules to initiate and maintain spermatogenesis[30]. LH is involved in the stimulation of leydig cells to produce testosterone for testicular function[30]. Testosterone is known to be critically involved in the development of sperm cells and any effect on its secretion may result from leydig cell dysfunction or testicular steroidogenic disorders[30].

Oxidative stress and low androgenic hormone levels have been identified as the major predisposing factors to testicular dysfunction and impairment of spermatogenesis in diabetic rats[28]. Interestingly, it's been shown that certain autocrine-paracrine signaling growth factors in the testis may be playing vital role in the proper functioning of the testis and hence, male reproduction[31,32]. One family of growth factors that have been incriminated is the insulin growth factors family (insulin, IGF-1 and IGF-2)[31]. Recent data have suggested that certain cell types within the testis may be a source of IGF production and that the synthesis of these local IGFs may function via autocrine-paracrine action within the testis to regulate various aspects of testicular development and function, which could be modulated by the level of insulin secretion[31-33].

Our results support these findings, especially after the administration of ALX to induce diabetic conditions. The mechanisms of action of DAE in ameliorating diabetic and testicular lesions in diabetic rats may be associated with pharmacological activities of its phytochemical constituents such as those in antioxidant activities[34]. Androgenic effects may also be mediated by phytochemical constituents of DAE, such as flavonoids[10,11,13], phenols, anthraquinones[13,31], saponins, tannins[31], alkaloids and phytosterols[13,31]. All of these phytochemicals were identified and confirmed to be present in *D. arborea* leaf extract in our preliminary phytochemical screening study[25]. This study has been able to scientifically confirm and unravel the hypothetic ethnomedical beliefs in most developing countries that *D. arborea* extracts may have anti-diabetic activity when administered at certain pharmacological relevant doses.

In conclusion, our study has been able to demonstrate the antidiabetic potential of ethanolic leaf extract of *D. arborea* in ameliorating the diabetes-induced hyperglycemia and oxidative stress-mediated testicular disorders in ALX-induced diabetic rats. Thus, it has antioxidant and hypoglycemic activities in ALX-induced diabetic rats. DAE was also capable of ameliorating dose dependently testicular endocrine disorders due to oxidative stress-mediated testicular damage in diabetic rats. The 300 mg/kg/day (EC_{50}) in this experiment was found to be relatively non-toxic and therapeutically effective against testicular and hormonal derangements. These

protective effects could also be attributed to the phytochemical constituents of DAE such as saponins, flavonoids, alkaloids, anthraquinone, sterols, and tannins, which may have supported its prospect to be used for prevention and treatment of diabetes-induced endocrine and testicular disorders in diabetic patients.

Conflict of interest statement

We declare that we have no conflict of interest.

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