ORIGINAL ARTICLE

Evaluation of the anti-diabetic effects of *Commelina diffusa* on testicular tissue of male Wistar rats

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Abstract

Background: Diabetes mellitus is a metabolic illness defined by elevated blood sugar levels caused by insulin deficit or resistance, as well as structural and functional problems in several organs. *Aim and Objectives*: This study assesses the efficacy of *Commelina diffusa* Extract (CDE) on the reproductive functions of diabetic male Wistar rats. *Material and Methods:* Thirty mature male Wistar rats were grouped into five groups of five rats each. Group 1: Normal control group; Group 2: High Fat Diet (HFD) + Streptozotocin (STZ); Group 3: HFD + STZ + 300mg/kg CDE; Group 4: HFD + STZ + 600 mg/kg CDE; Group 5: HFD + STZ + 1200 mg/kg CDE. All treatments were carried out for 21 days. On the 22nd day of the experiment, after an overnight fast, the experimental rats were sacrificed via cervical dislocation, and the testicular tissues were collected for biochemical, hormonal, and semen quality examination. The study found a substantial drop ($p < 0.05$) in antioxidant activity, male sex hormones, and sperm quality. Treatment with CDE significantly repaired the damage caused by diabetes. *Conclusion*: CDE is efficient in reducing oxidative stress, increasing antioxidant defense, improving reproductive hormone balance, and preserving male reproductive health in the face of poor metabolic circumstances.

Keywords: testis, hypoglycemic agent, oxidative stress

Introduction

Diabetes Mellitus (DM) is a complicated metabolic condition defined by high blood sugar levels [1]. It is approximated that 240 million individuals worldwide have undiagnosed diabetes, with approximately half of the adult population uninformed of their illness [2]. This illness affects all regions and is growing at an alarming rate worldwide.

Diabetes is marked by impaired insulin sensitivity or scarce insulin production, as well as abnormalities in glucose, lipid, and protein metabolism. Persistent hyperglycemia causes a number of metabolic problems, including non-enzymatic protein glycation, elevated generation of Reactive Oxygen Species (ROS). DM is frequently asymptomatic, which means that people may be unaware of their illness until they suffer organ damage as a result of chronic disease [3].

A high-fat diet is one in which fats account for a considerable proportion of daily calories, frequently surpassing recommended amounts. The buildup of extra fat is the result of an energy imbalance caused by a variety of circumstances, including the

ingestion of energy-dense meals [4]. Such high-calorie diets are a key factor to the rising incidence of obesity and related health issues [5]. Diets high in fructose and fat have been identified as contributors to the initiation of metabolic disorders such as obesity, diabetes, and dyslipidemia [6].

Diabetes management without complications or side effects remains a big problem in the medical industry [7]. Diabetes care is heavily influenced by traditional medicine, with over 1,200 plant species being used worldwide. Approximately 30% of these plants are primary sources of medicinal compounds for diabetes and other medical disorders [8]. Herbal medicine has received a lot of interest in recent years since it has few or no negative effects [9]. Natural diabetic solutions are very advantageous since they provide excellent therapy without causing negative side effects or toxicity.

Commellina diffusa has long been used as a medicinal herb. This pantropical species, often known as the climbing or spreading dayflower, is a herbaceous plant from the *Commelinaceae* family [10]. *C. diffusa* is native to tropical and subtropical climates and has traditionally been used as a diuretic, blood clotting agent, anti-poison, and cardiac tonic [11]. It has been used to remedy several of ailments throughout Asia, Africa, and the Americas, including urinary tract infections, respiratory infections, diarrhea, piles, and inflammation of the conjunctiva [12]. The plant's leaves and stems have been used to cure a range of conditions, including abscesses, boils, malaria, bug and snake envenomation, swelling (edema), inflammation of the larynx, pharynx, acute inflammation of the tonsils, middle ear infection, and epistaxis. [11]. Recent studies indicate that the plant may have anti-diabetic potential [13]. However, despite its intriguing promise for diabetes therapy, the mechanisms of action and influence on testicular health are little known. This study aims to look into the anti-diabetic benefits of *Commelina diffusa* on testicular tissue.

Material and Methods

This research was conducted in the Department of Anatomy, College of Health Sciences, Delta State University, Abraka, Nigeria. The Ethics Committee of the Faculty of Basic Medical Science, Delta State University, requested and granted permission to conduct this research project. All animal experiments followed the Institutional Animal Ethics Committee's (IAEC) recommendations.

This study used thirty (30) male Wistar rats. Under conventional laboratory settings, the rats were maintained in plastic cages with netted covers for ventilation. All rats were supplied regular meal and water. Before the trial began, the rats were permitted to adapt to their surroundings for 7 days.

Research Design

Thirty mature male Wistar rats were organized into six groups consisting of five animals each. The animals were organized as follows:

Group 1: Control

Group 2: High fat diet (HFD) + Streptozotocin (STZ)

Group 3: HFD + STZ + 300 mg/kg *Commellina diffusa* extract (CDE)

Group 4: HFD + STZ + 600 mg/kg CDE

Group 5: HFD + STZ + 1200 mg/kg CDE

Preparation of CDE

The fresh leaves (1000 g) of *C. diffusa* were dried naturally at ambient temperature and ground with a blender. Three grams of crushed leaves were treated

Table 1: Formula of a fat-rich diet

Soybean meal (g/100 g)	Casein (g/100 g)	Mountain flour (g/100 g)	Sodium cholate (g/100 g)	Refined wheat (g/100 g)	$CaHCO_3$ (g/100 g)	Fructose (g/100 g)	Vitamin (g/100 g)
6.5	11.0	0.6	0.2	40.0	1.0	15.7	2.0

in 5% Dimethyl Sulfoxide (DMSO). Following roughly a day of standing, the solution was filtered and stored in an airtight container with proper labeling.

Preparation of HFD

A diet rich in fat was formulated according to the following constituents presented (Table 1).

Induction of diabetes

Blood glucose levels were measured in all animals prior to streptozotocin/citrate buffer therapy. The control and experimental groups had identical blood glucose levels. The control group received merely 0.1 mL citrate buffer, but the diabetic group received 40 mg/kg STZ for five days. Diabetes was established by measuring blood glucose concentrations (over 250 mg/dl) on a weekly basis with a glucometer.

Sample collection

The experimental animals were sacrificed via cervical dislocation on the 22nd day of the trial, after a nocturnal fasting period. Each rat was put on its dorsal surface, and a laparotomy was done to reveal the internal organs. The testes were removed, and samples were taken and stored in sterile, labeled containers.

Determination of antioxidants in testicular tissue

Testicular antioxidant levels such as Malondialdehyde (MDA), reduced Glutathione (GSH), Glutathione Peroxidase (GPx), Total Antioxidant Capacity (TAC), Nitric Oxide (NO), Superoxide Dismutase (SOD), and Catalase (CAT), were measured spectrophotometrically. The serum samples spun at 3000 rpm for 10 minutes to extract the supernatant. To perform each test, the relevant dilutions were prepared as per the kit instructions and 100 µL of the sample was added to pre-coated microplates. After adding particular enzyme conjugates and substrates, the plates were incubated at room temperature for 30-60 minutes. The absorbance was measured with an absorbance reader at the wavelengths prescribed for each experiment (450 nm). Standard curves were created to measure the levels of each antioxidant marker, and all experiments were repeated to confirm accuracy.

Determination of serum hormone concentrations

ELISA kit was used to quantify the amounts of testosterone, Luteinizing Hormone (LH), Follicle-Stimulating Hormone (FSH), and Anti-Müllerian Hormone (AMH). The optical densities of the samples were measured using an ELISA reader with wavelengths set to 450 and 630 nm. The hormone concentrations were determined using a previously described technique [14].

Semen analysis

A piece of the minced epididymis was preserved in a 10% formalin / PBS solution (pH 7.4) and placed

on a hemocytometer for sperm quantification under a light microscope. Another fraction was put on a glass slide and examined under the same microscope for sperm motility. To evaluate morphology, a portion of sperm suspension was stirred with an equivalent volume of 1% eosin and evaluated at 200× magnification. Morphological anomalies, such as headless, tailless, curved head, and coiled tail sperm, were seen. To determine viability, a drop of sperm sample was combined with two drops of 1% eosin for 30 seconds, then incubated with three drops of 10% nigrosin. Dead sperm looked pink and were tallied in each field of vision. All analyses were carried out at 200× magnification. Sperm count, motility, and viability were expressed as percentages following published techniques [15].

Statistical analysis

All values from the studies were combined and statistically analyzed with the Statistical Package for Social Sciences (SPSS) analytic software, version 23.0. Results were provided as mean ± standard deviation.

Results

In rats treated with HFD+STZ, MDA levels significantly elevated ($p < 0.05$), and GSH, GPx, CAT, TAC, and NO activity reduced ($p < 0.05$) compared to control (Table 1). In contrast, groups that were induced with a HFD and STZ but received graded doses of CDE (HFD+STZ+CDE) demonstrated a marked ($p < 0.05$) reduction in MDA activity, as well as a marked ($p < 0.05$) elevation in GSH, GPx, SOD, CAT, TAC, and NO levels relative to the HFD+STZ group (Table 2).

Male Wistar rats on HFD+STZ had significantly lower testosterone, FSH, LH, and AMH concentrations compared to the control group (Table 3).

Table 2: Effect of *Commenlina diffusa* on testicular antioxidant induced with diabetes and high fat diet

Groups	MDA (µm)	GSH (µm)	GPx (µm)	SOD (U/mm)	CAT (µm)	TAC (mM)	NO (µmol/L)
Neutral control	8.793 ± 0.129	3.1867 ± 0.055	2.973 ± 0.058	0.258 ± 0.040	10.230 ± 0.06	0.055 ± 0.006	12.410 ± 0.066
HFD+STZ	59.168 ± 0.100*	2.14 ± 0.059*	2.285 ± 0.065*	0.118 ± 0.053	6.486 ± 0.284*	0.025 ± 0.003*	6.8433 ± 0.108*
HFD+STZ+300 CDE	22.001 ± 0.063*a	8.126 ± 0.072*a	5.086 ± 0.051*a	0.665 ± 0.049*a	10.673 ± 0.053*a	0.067 ± 0.005*a	15.223 ± 0.065*a
HFD+STZ+600 CDE	21.431 ± 0.094*ab	8.458 ± 0.05*ab	5.296 ± 0.068*ab	0.848 ± 0.065*a	10.883 ± 0.064*a	0.084 ± 0.004*ab	15.498 ± 0.069*ab
HFD+STZ+1200 CDE	20.825 ± 0.105*abc	8.696 ± 0.069*abc	5.926 ± 0.122*abc	0.534 ± 0.473*ac	11.198 ± 0.073*abc	0.102 ± 0.006*abc	16.083 ± 0.070*abc

*Results are presented as mean ± SD, HFD= High Fat Diet, STZ= Streptozotocin, CDE= Commenlina diffusa extract, MDA = Malondialdehyde, GSH = Glutathione, GPx = Glutathione Peroxidase, SOD = Superoxide dismutase, CAT = Catalase, TAC = Total antioxidant capacity, NO = Nitric oxide *=significantly different from neutral control, a= significantly different from HFD+STZ, b= significantly different from HFD+STZ+300CDE, c = significantly different from HFD+STZ+600CDE*

Table 3: Effect of *Commenlina diffusa* on sex hormones in male wistar rat induced with diabetes and high fat diet

Groups	Testosterone (nmol/L)	FSH (mU/mL)	LH (mU/mL)	AMH (ng/mL)
Neutral Control	10.696 ± 0.089	5.555 ± 0.093	3.201 ± 0.063	20.293 ± 0.133
HFD+STZ	5.970 ± 0.09*	4.393 ± 0.068*	2.671 ± 0.096*	0.056 ± 0.027*
HFD+STZ+300 mg/kg CDE	15.168 ± 0.073*a	7.193 ± 0.631*a	4.108 ± 0.085*a	25.271 ± 0.110*a
HFD+STZ+600 mg/kg CDE	17.280 ± 0.155*ab	8.210 ± 0.093*ab	6.035 ± 0.103*ab	30.221 ± 0.122*ab
HFD+STZ+1200mg/kg CDE	20.281 ± 0.103*abc	9.241 ± 0.114*abc	7.285 ± 0.162*abc	60.353 ± 0.187*abc

*Results are presented as Mean ± SD, HFD= High Fat Diet, STZ= Streptozotocin, CDE= Commenlina diffusa extract, *=significantly different from neutral control, a= significantly different from negative control, b= significantly different from HFD+STZ+300CDE, c = significantly different from HFD+STZ+600CDE*

Rats given HFD+STZ with graded dosages of CDE showed a substantial ($p < 0.05$) rise in all measured sex hormones in comparison to HFD+ STZ-only group. Furthermore, CDE's influence on sex hormone levels was dose-dependent, with higher doses having stronger effects and the 1200 mg/kg dosage exhibiting the greatest significant rise (Table 3). Wistar rats subjected to HFD+STZ had significantly reduced sperm count and motility ($p < 0.05$) (Table 4). In contrast, rats induced with HFD+STZ and treated with graded dosages of CDE (HFD+STZ+CDE) showed substantial ($p < 0.05$) improvement in sperm count, viability and motility. Furthermore, the effect of CDE on sperm parameters was dose-dependent, with larger dosages producing more significant changes (Table 4).

Table 4: Effect of *Commenlina diffusa* on semen parameters in male Wistar rat induced with diabetes and high fat diet

Groups	Sperm count $\times 10^6$ (cells/mL)	Sperm Viability (%)	Sperm Motility (%)
Neutral Control	32.166 ± 5.307	60.193 ± 0.072	50.711 ± 0.065
HFD+STZ	8.666 ± 3.777*	33.876 ± 3.064*	31.556 ± 2.306*
HFD+STZ+300 mg/kg CDE	45.666 ± 4.179*a	60.383 ± 0.133a	66.050 ± 0.139*a
HFD+STZ+600 mg/kg CDE	64.500 ± 4.460*ab	65.276 ± 0.151*ab	67.290.172*a
HFD+STZ+1200 mg/kg CDE	60.353 ± 0.187*abc	80.500 ± 3.449*ab	66.123.235*ab

*Results are presented as Mean ± SD, HFD= High Fat Diet, STZ= Streptozotocin, CDE= Commenlina diffusa extract, *=significantly different from neutral control, a= significantly different from negative control, b= significantly different from HFD+STZ+300CDE, c = significantly different from HFD+STZ+600CDE*

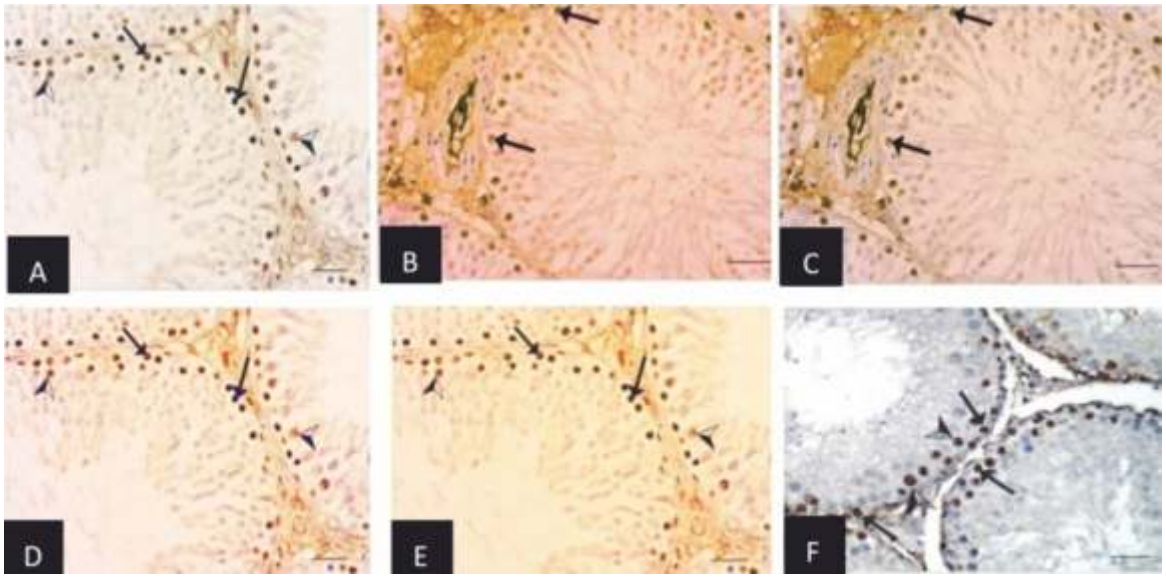

Figure 1: Immunohistochemical staining of testes. Session (A) Neutral control shows few visible PCNA-positive cells (brown nuclei) indicating limited cell proliferation (B) Positive control [High Fat Diet + Streptozotocin] shows increased PCNA-positive cells, marked by arrows, reflecting a higher level of cell proliferation due to treatment with metformin (C) High Fat Diet + Streptozotocin + 300 mg/kg of *C. diffusa* extract shows several PCNA-positive cells, though possibly fewer than in the positive control, suggesting a moderate level of proliferation with the lower dose of *C. diffusa* extract (D) High Fat Diet + Streptozotocin + 600 mg/kg of *C. diffusa* extract shows slightly increased number of PCNA-positive cells (E) High Fat Diet + Streptozotocin + 1200 mg/kg of *C. diffusa* extract shows increased PCNA-positive cells, indicating the dose-dependent effect of *C. diffusa* extract in promoting cell proliferation (F) Negative control [High Fat Diet + Streptozotocin] shows few PCNA-positive cells, indicating low cell proliferation. Arrows indicate the presence of spermatogonia.

Discussion

In this work, STZ-induced diabetes caused a considerable increase in lipid peroxidation within testicular tissues, as evidenced by higher MDA levels. Furthermore, diabetic rats' testes showed a reduction in critical cellular antioxidant defenses such as GSH, CAT, SOD, NO, and TAC. These findings are consistent with earlier studies indicating that hyperglycemia-induced testicular injury in diabetic rats is caused by high oxidative stress or downregulation of antioxidant mechanisms [16-18].

Diabetic rats treated with CDE showed significantly lower MDA activity ($p < 0.05$) and higher levels of GSH, GPx, SOD, CAT, TAC, and NO compared to untreated rats. The significant increases in oxidative stress indicators and antioxidant enzyme activities indicate that CDE has substantial antioxidative characteristics, which contribute to the reduction of oxidative damage and the promotion of cellular function. These findings are similar to reports which show efficacy of antioxidants in alleviating oxidative stress [10, 11, 19, 20].

The considerable drop in MDA levels in the CDE-treated group suggests a significant reduction in oxidative stress and lipid peroxidation, emphasizing CDE's antioxidant capability. The significant rise in GSH levels shows an improvement in the body's antioxidant defense mechanism, implying the restoration or over expression of endogenous antioxidant capability. Furthermore, increased GPx and SOD activity suggests a stronger enzymatic antioxidant defense, allowing for more effective neutralization of ROS [21].

The increased CAT activity supports CDE's involvement in strengthening antioxidant defenses by promoting the breakdown of hydrogen peroxide, therefore alleviating oxidative stress. The rise in TAC levels suggests an increase in total antioxidant capacity, which aids in the preservation of cellular redox equilibrium. Furthermore, the considerable increase in NO levels indicates better endothelial function [22] and a higher anti-inflammatory response in the CDE-treated group. The observed dose-dependent effects, in which higher CDE concentrations resulted in greater reductions in MDA levels and further increases in GSH, GPx, SOD, CAT, TAC, and NO activities, indicate that CDE has a dose-responsive protective and antioxidative effect against diabetes-induced oxidative damage.

STZ-induced diabetic rats showed substantial decreases in all tested sex hormones, including testosterone, FSH, LH, and AMH, indicating compromised sexual and reproductive function. This observation is consistent with prior study findings [23-26]. Hyperglycemia has been shown to induce testicular injury, alter spermatogenesis, and reduce sperm count by producing excessive ROS in reproductive organs such as the sperm, epididymis, and testes, ultimately leading to apoptosis in both somatic and germ cells [27-28].

Diabetic rats given with graded dosages of CDE showed significant elevation ($p < 0.05$) in testosterone, FSH, LH, AMH levels compared to untreated diabetic rats. The significant elevation in testosterone shows that CDE increases androgenic activity, may be by boosting testicular function or enhancing general metabolic health. The increase in FSH and LH levels suggests that the Hypothalamic-Pituitary-Gonadal (HPG) axis is better regulated, most likely due to lower metabolic stress and greater endocrine signaling caused by CDE. Furthermore, the rise in AMH levels lends credence to CDE's involvement in enhancing gonadal function and maintaining reproductive hormone balance. The dose-dependent rise in hormone concentrations in CDE-treated rats shows that larger dosages provide greater advantages, most likely by more effectively combating oxidative stress, boosting metabolic processes, and improving endocrine regulatory pathways.

In this study, sperm quality, including count, viability, and motility, was significantly decreased in STZ-induced diabetes rats, which is consistent with prior research [29-30]. This decrease in sperm quality is a result of oxidative stress, hormonal abnormalities, and testicular injury.

However, diabetic rats treated with CDE showed considerable improvements in sperm parameters, indicating that CDE has a profound influence on male reproductive health, particularly under the metabolic stress caused by diabetes. The rise in sperm count shows that CDE may boost spermatogenesis, presumably by reducing oxidative damage to the testes and promoting a more favorable hormonal milieu for sperm formation. The increase in sperm viability, which measures the proportion of living and functioning sperm, implies that CDE

not only increases sperm number but also boosts sperm health and functional integrity, both of which are critical for fertility. Furthermore, the considerable increase in sperm motility seen after CDE therapy suggests that it may enhance sperm energy metabolism and structural integrity. This improvement is most likely due to CDE's anti-oxidant qualities, which assist minimize oxidative stress and preserve sperm cells, resulting in improved motility.

Conclusion

The findings of this study showed that HFD and diabetes increase oxidative stress, disturb hormonal balance, and have a detrimental impact on sperm quality. The findings also show the potential therapeutic effects of CDE in lowering oxidative stress, improving antioxidant defenses, restoring reproductive hormone balance, and protecting male reproductive health under poor metabolic situations. Notably, CDE's dose-dependent efficiency demonstrates its enormous potential as a natural antioxidant and protector against oxidative stress and concomitant reproductive dysfunctions.

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