Reducing Sugar, Alkaloid and Tannin from *Dryopteris dilatata* Fractions Modulates Diabetogenic and Oxidative Stress Activity on Alloxan Induced Diabetic Rats.

ABSTRACT

Active components in medicinal plants possess therapeutic indications in disease states.

Aim: The present investigation evaluated the activity of fraction from *Dryopteris dilatata* leaves modulating diabetogenic and oxidative stress activity in alloxan-induced diabetic Rats.

Method: Seventy-two male (135-140)g wistar rats divided into two groups of thirty-six rats each for oral glucose tolerance test and diabetic study. Diabetic induction and oral glucose test (OGTT) was done using standard methods. Each group was divided into six sub-groups (n-6). Group A was normal control, group B diabetic control, group C received metformin 50 mg/kg, group D reducing sugar fraction 800 mg/kg, group E alkaloid fraction 800 mg/kg and group F tannin fraction 800 mg/kg once for OGTT and daily throughout the treatment period (15 days) for diabetic study. Their glucose level was taken at interval of hours for OGTT and five days interval for diabetic group. We assessed the levels of lipid profile (TC, TG, LDL and HDL), lipid peroxidation, endogenous antioxidants in the brain and testis.

Results: Fractions of *Dryopteris dilatata* caused significant reduction in body weight and blood glucose levels in experimental rats, decreased the levels of TC, TG, LDL, increased HDL, reduced levels of MDA, elevated CAT, GSH and SOD in the brain and testis.

Conclusion: The observed results in this study connotes that fractions of *Dd* could be used in amelioration of diabetes and its associated complications by reducing bad cholesterol (TC, TG and LDL), increasing good cholesterol (HDL), attenuating the activity of antioxidants in diabetic condition.

Key words: Alloxan, Diabetes, *Dryopteris dilatata*, Lipid Profile, OGTT, Antioxidants

Graphical Abstract

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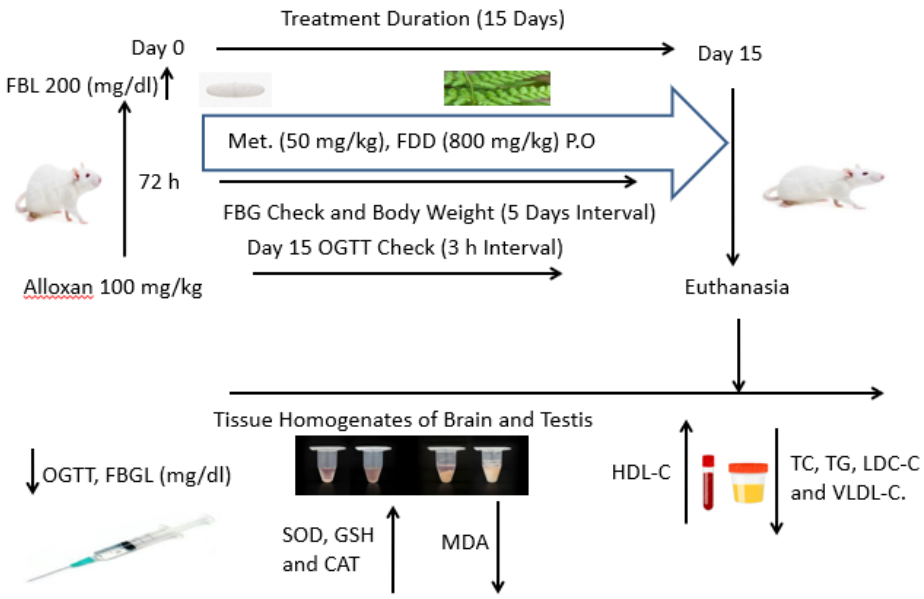
Only In vivo method explained here



Introduction

Hyperglycemia accompanied by macro and micro-vascular damages in human system is attributed to metabolic disorder that result from the inability of living system to produce or utilize insulin, in this defect the pancreas is either not producing insulin due to the autoimmune attack or other attack from infection and environmental toxins or its warn out through it being over worked truing to meet the body's demand for insulin when there is continuous rise in blood glucose when there is tissue resistance to insulin [1]. Globally diabetes has been associated with increased morbidity and mortality due to its associated complications in the cardiovascular and

neuronal system all of which negatively affect the quality of life [2]. Most disease conditions have involvement in the pathogenesis of diabetes mellitus, as viruses and other infectious diseases, environmental toxins can attack and destroys the pancreatic beta cells rendering it unable to produce enough insulin for the body's metabolic demands and also affect the cells causing insulin resistance, the result of insulin deficiency and or resistance affects the metabolism of carbohydrates fats and proteins as it prevents its mobilization into tissues [3].

Metabolic disorders affecting humans have been managed since time immemorial with herbal remedies as medicinal plants use in the health care industry has gained popularity due to significance advantage over orthodox remedies in the treatment many health conditions in all over the world since it is readily available, less expensive and reduced or no deleterious effect, and has been a good source for the development of new drugs as most orthodox remedies are synthesized from herb due to promising phytochemicals that possess diverse therapeutic potentials in the treatment of several health conditions [4]. Global statistics shows that more than of the world's population depends on herbal remedies for their everyday health needs and a large amount of these remedy is gotten from plants and their various extracts which contain constituents that have therapeutic activity which result from plant extracts ability ameliorate many health defects without associated harmful effects on organs and systems of individuals compared to orthodox remedies [5].

Convectional remedies used in the management of diabetes mellitus which includes oral hypoglycemics and several types of insulin are of great therapeutic benefit despite their undesirable effects, but since diabetes has become a global health problem with a high rate of increase in developed countries, greater number of people being affected with the rural dwellers not left out has affected the economic and health sector [6]. The use of herbal therapies in the management of several ailments has gained global attention in recent times due to its numerous therapeutic potentials, wide margin of safety which has led to the use of medicinal plants with phytochemicals that possess therapeutic potency on laboratory animals as experimental tools for their efficacy and safety on several ailments of which diabetes mellitus is one of such conditions [7].

The list of medicinal plant is with therapeutic benefits is inexhaustible as a great number of plant species have been recorded with considerable constituents that are active against diabetes mellitus and its associated complications on several organs [8]. Anti-diabetic medicinal plants possess blood glucose reducing constituents that can serve as lead targets for the production of pharmaceutical agents and supplements for a wide variety of health conditions [9]. *Dryopteris dilattata* is among such medicinal plant where its leaves possess phytochemicals with anti-diabetic activity [10].

Materials and Methods

Drugs and chemicals

Alloxan, thiobabituric acid, trichloroacetic acid, adrenaline, all other drugs analytical kits and chemicals used for the experiment were gotten from Sigma-Aldrich, St Louis, Mo, USA. Accu-check Active Glucometer from Roche diagnostic, Mannhein Germany. The chemicals were of analytical grade.

Plant Collection, Identification and Extraction

The leaves of *Dryopteris dilatata* were collected from its habitat in Olomoro community in Isoko South, Delta state Nigeria. Some was identified by Dr Erherhi of the Department of Botany and authenticated in Forestry Research Institute of Nigeria, Ibadan with herbarium number, FHI 110338. The plant crude extraction method was adapted from Akpotu *et al*., [11]. The leaves were air-dried followed by extraction after 72 hours. The extraction was done using ethanol as the first solvent of choice, thereafter the residue was further used crude extract for ethyl acetate following same procedure. After which both concentrated solvent was used for vacuum liquid chromatography where using silica gel mesh 60-120 (particle size), where fractions of reducing sugar, alkaloid and tannin was obtained, The fractions obtained were concentrated to dryness with the aid using a rotary evaporator and preserved in a refrigerator until use. Distilled water used to dissolve the fractions to obtain the required dose of 800 mg/kg.

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Animals

A total of seventy-two [72] adult Wister rats weighing between 120-150 g divided into set: Oral Glucose Tolerance Test (OGTT) and alloxan-induced diabetic (Diabetic) were used for the study The Wister rats were purchased from the animal house of the Department of Pharmacology and Therapeutics, University of Nigeria Enugu campus. The animals were allowed to acclimatize for two weeks before the commencement of the experiment, exposed to 12 h light and 12h dark periods and were fed with standard diet (growers mash) and water *ad libitum* for the duration of the experiment. The study protocol was carried out in accordance with the ethical standards of NIH guidelines (revised 1978).

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Oral glucose tolerance test (OGTT)

The oral glucose test was performed according to Rocha *et al.*, [12] with slight modification. The Oral Glucose Tolerance Test (OGTT) was performed on overnight fasted normal rats. Glucose (2 g/kg) was fed 30 min after pre-treatment with fraction of *Dryopteris dilatata*.

Group *A*-Normal control rats (normal); Group *B*- Glucose control rats; Group *C*- Glucose + Metformin (50 mg/kg), Group *D*- Glucose rats +Reducing Sugar Fraction (800 mg/kg), Group E- Glucose + Alkaloid Fraction (800 mg/kg) and Group F- Glucose +Tannin Fraction (800 mg/kg). The drug and plant fractions was administered using oral gastric cannula, blood glucose levels were measured at 1 hour, 3 hours, 6 hours, 12 hours and 24 hours after glucose administration, using a commercial glucometer (Accu- check Active, Roche diagnostic, Mannhein Germany).The control groups (normal and glucose) received distilled water in the place of the fractions and standard anti-diabetic drug.

Diabetes Induction

Diabetes was induced in Wistar rats by single intra-peritoneal injection of 100 mg/kg of Alloxan monohydrate dissolved in 0.9 M sodium chloride buffer (pH 7) after an overnight fast using method of Akpotu *et al.* [11] with slight modification,, after the induction of diabetes the fasting blood glucose level was measured after 72 h. Fasting blood glucose level of 200 mg/dl and above were considered diabetic and were selected for the study.

Treatment Design

Normal control + distilled water; diabetic control + distilled water; diabetic + metformin (50 mg/kg), diabetic + reducing sugar fraction (800 mg/kg), diabetic + alkaloid fraction (800 mg/kg) and diabetic +tannin fraction (800 mg/kg). The drug and plant fractions was administered daily using oral gastric cannula, fasting blood glucose levels were measured at five days' interval for fifteen days along body weight determination, using a commercial glucometer (Accu- check Active, Roche diagnostic, Mannhein Germany). The control groups (normal and diabetic) received distilled water in the place of the fractions and standard anti-diabetic drug.

Collection and Preparation of Samples

At the end of the treatment duration, blood glucose levels were determined and the rats were euthanized followed by whole blood collection through cardiac puncture. Organs such as testis, brain were excised and stored appropriately for biochemical analysis.

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Tissue homogenate preparation

The brain and testis of the experimental rats were homogenized using buffer solution of 10 % (Tris-HCL, KCL of pH 7.4), cold centrifuged at 10,000 rpm for 3 min at 4°C. The supernatants were collected and stored for biochemical analysis.

Lipid profile analysis

Estimation of total cholesterol activity

Equal amounts of the reagents (500 µl) and samples (5 µl) was pipetted at room temperature into labeled tubes (Blank, Standard and Sample), while distilled water was added to the blank in place of the sample. The reagents were mixed and the tubes were allowed to stand for five (5) minutes at 37 ^{0}C. Thereafter, the absorbance of the samples and the standard were read using spectrophotometer at 500 nm against the blank reagent [13, 14].

Estimation of high density lipoproteins activity

Same quantity of the reagents and samples were pipetted at room temperature, into labeled tubes. Missed and allowed to stand for ten (10) minutes at room temperature, after which they were centrifuged for ten (10) minutes at 4000 rpm, and the clear supernatant was separated within two (2) hours. Then, the absorbance of the samples and the standard were read using spectrophotometer at 500 nm [15, 14].

Estimation of triglycerides activity

This was done by pipetting the reagent (0.5 ml) and sample (5 µl), Standard (5 µl) and reagent (500 µl) at room temperature into labeled test tubes,. They were mixed and the tubes were incubated for 5 min. at 37 ^{0}C. The absorbance of the samples and standard were read at 500 nm using a spectrophotometer [15, 14].

Determination of VLDL and LDL cholesterol by calculation:

Very low density lipoprotein (VLDL) was obtained by calculation using the formula:

TG/5 (mg/dl).

Low density lipoprotein (**LDL**) was calculated using the formula:

LDL = TC - TG/5 (mg/dl) [14].

Estimation of the activity of lipid peroxidation (MDA)

The tissue supernatants of the brain and testis were estimated for pro-oxidant activity measuring the lipid peroxidation end product (Thiobabituric acid reactive substance) according to Matzkin *et al.*, [16] methods, and expressed as gmol MDA/g.

Estimation of antioxidants activity

Superoxide dismutase (SOD)

The activity of superoxide dismutase was carried out according to protocol of Misera [17], it was done following the principle of inhibiting adrenaline autoxidationusing sodium carbonate buffer (pH 10.7). Adrenaline autoxidation kinetics in tissue supernatant was monitored for 3 min, while

the absorbance was read at 495nm at interval of 60 and 240 seconds expressing the activity in U/mg protein.

Reduced glutathione activity (GSH)

Reduced glutathione activities in the brain and testis were estimated using Singh *et al*., [18] methods with the aid of Elman reagent. Supernatants of both tissues was deproteinized by adding 1 ml of Trichloroacetic acid, centrifuged. Thereafter the supernatant was added to 0.75 ml of sodium phosphate buffer (0.1 M, pH 7.4) and mixed with 2 ml of 51, 51-Dithios-nitrobenzoic acid. The spectrophotometer (752N INESA, China) absorbance was read at 412 nm in 5 min (μM GSH/mL).

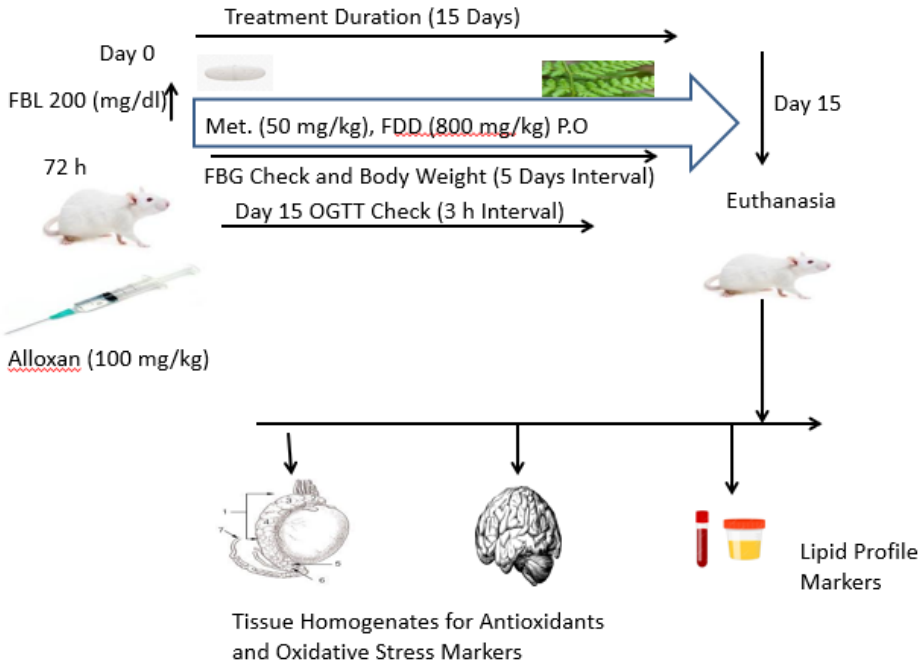
Catalase (CAT)

Catalase levels were determined according to Göksel *et al*., [19] method. Catalase activity was measured with aid of a spectrophotometer (INESA 750 N, china) model at 405 nm absorbance level and enzyme activity measured in kU/mg protein for all tissue.

Statistical analysis

Data collected were expressed in mean ± SEM (Mean and standard error), analyzed using one-way analysis of variance (ANOVA) followed by post hoc (LSD) for multiple comparison and $p<0.05$ was considered statistically significant. Statistical analysis was performed using SPSS version 21 software.

Graphical Methodology



Results

After loading the rats with glucose there was significant ($p<0.05$) increase in the level of blood glucose in glucose loaded rats when compared to normal control rats (Table 1), but at the 3rd, 6th, 12th and 24th h rats pretreated with FDD (800 mg/kg) and Metformine (50 mg/kg) revealed a significant ($p<0.05$) decrease in glucose level when compared with the non-treated hyperglycemic rats (group B), however pretreatment with Metformin (50 mg/kg) and AFDD (800 mg/kg) showed a significant ($p<0.05$) reduction in blood glucose when compared with RSFDD and TFDD (800 mg/kg) pretreated rats (Table 1).

Table 1. Activity of fractions of *Dryopteris dilatata* on glucose level of glucose induced hyperglycemia.

Groups	Initial Gluc. Level (mg/dl)	0 Hr (mg/dl)	1 Hr (mg/dl)	3 Hrs (mg/dl)	6Hrs (mg/dl)	12Hrs (mg/dl)	24Hrs (mg/dl)
A	78.67±4.38	82.50±3.06	85.83±2.26	83.50±2.06	85.83±3.51	84.67±2.49	87.33±2.42
B	80.83±4.60	81.83±4.13	128.83±1.25[a]	124.67±1.26[a]	121.67±1.65[a]	117.67±1.41[a]	114.67±3.86[a]
C	74.33±2.17	75.33±2.06	119.00±0.58[a]	111.50±2.84[ab]	99.17±2.93[ab]	89.50±1.89[b]	78.17±1.01[b]
D	75.50±3.99	76.50±3.60	119.33±0.84[a]	108.50±2.93[ab]	95.50±1.12[ab]	89.17±1.42[b]	84.33±1.09[bcd]
E	75.50±3.53	76.33±3.49	118.17±1.01[a]	109.00±3.24[ab]	98.67±2.86[ab]	87.83±1.92[b]	79.50±1.26[b]
F	76.83±3.49	77.83±3.54	119.83±1.138[a]	112.00±2.65[ab]	102.00±3.53[ab]	93.00±1.71[b]	86.17±1.45[bcd]

Data are expressed as means ± SEM (n=6), [a]p<0.05 versus normal control, [b]p<0.05 versus hyperglycemic, [c]p<0.05 versus D + Met. (50 mg/kg) and [d]p<0.05 versus G + AFDD (800 mg/kg) using one-way ANOVA followed LSD post hoc test.

Abbreviations: A-Normal control, B-Diabetic control, C; Metformin, D-Reducing Sugar Fraction of *Dryopteris dilatata*, E-Alkaloid Fraction of *Dryopteris dilatata*, F-Tannin Fraction of *Dryopteris dilatata*.

Following induction of diabetes using alloxan monohydrate there was observed significant ($p<0.05$) reduction of body weight of diabetic compared to the normal control rats (Table 2), but treated rats showed a significant ($p<0.05$) elevation body weight compared to diabetic control rats, moreover, treatment with AFDD (800 mg/kg) revealed a significant ($p<0.05$) elevation in body weight compared to Met. (50 mg/kg), RSFDD and TFDD (800 mg/kg (Table 2)

Table 2. Activity of fractions of *Dryopteris dilatata* on body weights of alloxan-induced diabetic rats

Groups	Innitial	Day 0	Day 5	Day 10	Day 15

	Body weight (mg/kg)				
Normal control	135.13±4.44	144.50±2.34	151.12±2.34	133.89±26.87	165.90±2.23
Diabetic control	140.17±6.09 (N.S)	130.07±5.38	120.29±6.28 [a]	105.96±5.97[a]	94.09±4.41[a]
D + Met ()50 mg/kg	139.79±4.35	129.16±4.14	130.84±3.97 [b]	132.49±4.05[b]	134.08±4.05[b]
D + RSFDD (800 mg/kg)	137.40±5.21	127.85±5.03	128.34±4.80[b]	129.58±4.75[b]	130.55±4.72[b] [c]
D + AFDD (800 mg/kg)	137.21±3.49	124.57±4.29	125.88±4.16[b]	127.58±4.18[b]	129.75±4.05[b] [c]
D + TFDD	136.20±5.28	127.10±4.99	128.22±4.87[b]	129.29±4.89[b]	130.44±4.84[b] [c]

Data are expressed as means ± SEM (n=6), [a]p<0.05 versus normal control, [b]p<0.05 versus diabetic, and [c]p<0.05 versus D + Met. (50 mg/kg) using one-way ANOVA followed LSD post hoc test.

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Abbreviations: Met-Metformin, RSFDD-Reducing Sugar Fraction of *Dryopteris dilatata*, AFDD- Alkaloid Fraction of *Dryopteris dilatata*, TFDD- Tannin Fraction of *Dryopteris dilatata.*

After induction of diabetes using alloxan monohydrate there was significant (p<0.05) increase in glucose level of diabetic rats compared to the normal control rats (Table 3), but treated rats revealed significant (p<0.05) decrease in blood glucose levels compared to the diabetic control

rats, while treatment with AFDD (800 mg/kg) was observed with significant ($p<0.05$) reduction in blood glucose levels compared to Met. (50 mg/kg), RSFDD and TFDD (800 mg/kg) (Table 3)

Table 3. Activity of fractions of *Dryopteris dilatata* on glucose levels of alloxan-induced diabetic rats

GROUPS	Initial Glucose Level (mg/dl)	Day 0 (mg/dl)	Day 5 (mg/dl)	Day 10 (mg/dl)	Day 15 (mg/dl)
Normal control	76.83±2.70	85.00±2.16	81.83±3.167	84.17±2.98	82.00±5.29
Diabetic control	73.33±3.12	266.33±16.13[a]	313.83±12.27[a]	369.33±11.2[a]	410.83±8.68[a]
D + Met (50 mg/kg)	73.50±3.70	288.33±29.91[a]	253.00±28.23[b]	203.17.692 [b]	172.17±31.57[bd]
D + RSFDD (800 mg/kg)	78.83±3.88	274.17±18.24[a]	225.33±14.10[b]	165.17±7.54[b]	131.33±4.94[bd]
D + AFDD (800 mg/kg)	69.33±1.41	281.67±15.71[a]	229.50±15.56[b]	172.17±12.09[b]	116.83±6.03 [b]
D + TFDD (800 mg/kg)	83.00±2.94	286.17±10.90[a]	236.67±13.72[b]	182.50±2.91[b]	132.83±4.66[bd]

Data are expressed as means ± SEM (n=6), [a]$p<0.05$ versus normal control, [b]$p<0.05$ versus diabetic, and [d]$p<0.05$ versus D + AFDD. (800 mg/kg) using one-way ANOVA followed LSD post hoc test.

Abbreviations: Met-Metformin, RSFDD- Reducing Sugar Fraction of *Dryopteris dilatata*, AFDD- Alkaloid Fraction of *Dryopteris dilatata*, TFDD- Tannin Fraction of *Dryopteris dilatata.*

The levels of serum total cholesterol, tryglcerides, low density lipoprotein and very low density lipoprotein in diabetic rats were observed with a significant ($p<0.05$) increase compared to the normal control rats while the diabetic rats showed a significant ($p<0.05$) decrease in the levels of serum high density lipoprotein compared to the normal control. However, treated rats were observed significant ($p<0.05$) decrease in the levels of serum total cholesterol, triglycerides, low density lipoprotein and very low density lipoproteins compared to the diabetic control rats, while there was observed significant ($p<0.05$) increase in the levels of serum high density lipoproteins in the treated rats compared to the diabetic rats. But TFDD (800 mg/kg) had a significant ($p<0.05$) decrease in serum total cholesterol, Met. (50 mg/kg) had a significant ($p<0.05$) decrease in triglycerides levels, RSFDD (800 mg/kg) had a significant ($p<0.05$) increased in high density lipoproteins levels, AFDD (800 mg/kg) had a significant ($p<0.05$) decrease in low density lipoproteins levels while Met. (50 mg/kg) and TFDD (800 mg/kg) showed a significant ($p<0.05$) decrease in the levels of serum very low density lipoproteins compared to other treated groups respectively (Table 4).

Table 4. Activity of fractions of *Dryopteris dilatata* on lipid profile of alloxan-induced diabetic rats

GROUPS	**TC (mg/dl)**	**TG (mg/dl)**	**HDL (mg/dl)**	**LDL (mg/dl)**	**VLDL (mg/dl)**
Normal control	101.93±3.309	95.23±2.88	40.71±1.06	42.17±3.00	19.07±.575
Diabetic control	184.21±3.60^{a}	200.14±5.64^{a}	26.50±1.38^{a}	118.34±3.68^{a}	40.03±1.129^{a}
D + Met (50 mg/kg)	108.26±3.53^{bf}	101.23±2.86^{b}	40.79±1.110^{bd}	46.88±2.98^{be}	20.59±0.53^{b}

D + RSFDD (800 mg/kg)	123.19±10.07[bf]	149.19±12.79[bc]	47.69±2.19[b]	55.42±6.19[be]	26.94±2.84[bcf]
D + AFDD (800 mg/kg)	106.60±9.85 [bf]	113.55±11.82[bc]	40.09±1.42[bd]	43.79±11.75[b]	22.71±2.37[bcf]
D + TFDD (800 mg/kg)	111.58±3.07 [b]	106.35±3.86[bc]	45.55±1.32[bd]	45.27±2.66[be]	20.731±0.88

Data are expressed as means ± SEM (n=6), [a]p<0.05 versus normal control, [b]p<0.05 versus diabetic, and [d]p<0.05 versus D + AFDD. (800 mg/kg) using one-way ANOVA followed LSD post hoc test.

Abbreviations: Met- Metformin, RSFDD- Reducing Sugar Fraction of *Dryopteris dilatata*, AFDD- Alkaloid Fraction of *Dryopteris dilatata*, TFDD- Tannin Fraction of *Dryopteris dilatata.*

Activity of lipid peroxidation on the tests of alloxan-induced d experimental rats was significantly (p<0.05) elevated in diabetic rats compared to the normal control rats (Table 5), but treated rats revealed a significant (p<0.05) reduction in the levels of malondialdehyde (MDA) compared to the diabetic rats, however, the activity of MDA levels in rats treated with Met. (50 mg/kg) was observed with a significant (p<0.05) decrease compared to RSFDD, AFDD and TFDD (800 mg/kg)

The levels of antioxidants markers (SOD, CAT and GSH) in the testis of experimental rats was significantly (p<0.05) decreased in diabetic rats compared to the normal control rats (Table 5), while the levels of anti-oxidants markers was significantly (p<0.05) increased in treated rats compared to the diabetic rats, however, the activity of CAT levels in AFDD was signid=ficantly (p<0.05) increased compared to Me. (50 mg/kg), RSFDD and TFDD (800 mg/kg) treated rats, then the activity of SOD and GSH levels in Met. (50 mg/kg) was significantly (p<0.05) increased compared to RSFDD, AFDD and TFDD (800 mg/kg) treated rats (Table 5)

Table 5 Activity of fractions of *Dryopteris dilatata* on Oxidative stress and antioxidant biomarker of the testis of alloxan-induced diabetic rats (nM/mg protein)

GROUPS	MDA	CAT	SOD	GSH
Normal control	1.29±0. 10	74.01±6.35	19.47±0.65	58.62±1.06
Diabetic control	2.65±0.09[a]	24.81±4.78[a]	10.09±0.84[a]	37.75±2.01[a]
D + Mt. (50 mg/kg)	1.49±0.13[b]	58.95±5.29[bd]	19.75±4.38[b]	53.96±0.85[b]
D + RSFDD (800 mg/kg)	1.61±0.13[bc]	75.08±2.82[bd]	14.24±0.67[bc]	46.55±2.19[bc]
D + AFDD (800 mg/kg)	1.64±0.1[bc]	113.02±22.55[b]	17.33±0.95[bc]	49.88±2.57[bc]
D + TFDD (800 mg/kg)	1.59±0.10[bc]	66.92±8.55[bd]	13.75±0.36[bc]	40.63±1.97[bc]

Data are expressed as means ± SEM (n=6), [a]$p<0.05$ versus normal control, [b]$p<0.05$ versus diabetic, and [d]$p<0.05$ versus D + AFDD. (800 mg/kg) using one-way ANOVA followed LSD post hoc test.

Abbreviations: Met- Metformin, RSFDD- Reducing Sugar Fraction of *Dryopteris dilatata*, AFDD- Alkaloid Fraction of *Dryopteris dilatata*, TFDD- Tannin Fraction of *Dryopteris dilatata*

The activity of lipid peroxidation of diabetic rats was significantly ($p<0.05$) elevated compared to the normal control rats, while the treated rets was observed with a significant ($p<0.05$)

decrease in MDA levels compared to the diabetic control rats, whereas rats treated with Met. (50 mg/kg) showed a significant decrease in MDA levels compared to rats treated with RSFDD, AFDD and TFDD (800 mg/kg).

The levels of endogenous anti-oxidants (SOD, CAT and GSH) were significantly ($p<0.05$) reduced in diabetic rats compared to the normal control (Table 6), while treated revealed a significant ($p<0.05$) increase in the levels of endogenous anti-oxidants compared to the diabetic rats, however rats treated with Met. (50 mg/kg) showed a significant ($p<0.05$) increase in the levels of CAT and GSH compared to RSFDD, AFDD and TFDD (800 mg/kg), rats treated with AFDD (800 mg/kg) showed a significant ($p<0.05$) increase in the levels of SOD compared to Met. (50 mg/kg), RSFDD and TFDD (800 mg/kg) treated rats (Table 6).

Table 6 Activity of fractions of *Dryopteris dilatata* on Oxidative stress and antioxidant biomarker of the brain of alloxan-induced diabetic rats

GROUPS	**MDA**	**CAT**	**SOD**	**GSH**
Normal control	0.83±0. 14	62.41±4.23	24.87±0.99	44.49±1.58
Diabetic control	2.56±0. 09 [a]	26.32±2.35 [a]	11.43±0.51[a]	24.48±1.27[a]
Met. (50 mg/kg)	0.78±0. 26[b]	52.62±7.55[b]	20.78±1.11[bd]	40.29±2.13 [b]
D + RSFDD (800 mg/kg)	1.02±0. 26[b]	45.95±10.74[bc]	16.20±0.66[b]	32.84±3.49[bc]
D + AFDD (800 mg/kg)	1.39±0. 20[b]	51.45±10.53[bc]	22.49±0.68[bd]	41.50±1.48[bc]
D + TFDD (800 mg/kg)	1.18±0. 35 [b]	48.960±7.00[bc]	15.37±0.68[bd]	29.29±1.19[bc]

Data are expressed as means ± SEM (n=6), [a]$p<0.05$ versus normal control, [b]$p<0.05$ versus diabetic, and [d]$p<0.05$ versus D + AFDD. (800 mg/kg) using one-way ANOVA followed LSD post hoc test.

Abbreviations: Met- Metformin, RSFDD- Reducing Sugar Fraction of *Dryopteris dilatata*, AFDD- Alkaloid Fraction of *Dryopteris dilatata*, TFDD- Tannin Fraction of *Dryopteris dilatata.*

Discussion

Result from earlier research on diabetes using animal models has shown the efficacy of herbal remedies in hyperglycemic state [20]. In the present investigation alloxan was used intraperitonealy to induce diabetes into wistar rats. Hyperglycemic condition observed after alloxan administration in wistar rats is the ability of the mechanism of alloxan to destroy the beta producing cells of the pancreas through its cytotoxic action by generation of free radicals, this reduced or absolute deficiency of insulin can subsequently lead to high blood glucose levels [21]. The observed significant elevation of blood glucose observed in the diabetic induced experimental rats was reduced to near normal after daily administration of several fractions of Reducing sugar, Alkaloid and Tannin of Dd for the duration of the experiment duration. Our observed result correlates with several other medicinal plants studies in experiment diabetes using alloxan as a vehicle for induction [22]. Efforts of oral hypoglycemics to improve the capacity of glucose tolerance are among the mechanism through which diabetes can be managed. In this study pre-treatment with plant fractions before glucose administration in rats revealed the glucose tolerance capacity of the pancreas to release insulin on in wistar rats this capacity of the plant fractions to mobilize insulin release and consistently decrease blood glucose to normal levels shows the potency of the plant fraction in glucose tolerance. It could be that the fractions of *Dryopteris dilatata* leaf possess increased glucose utilization effect which implies that it could improve glucose homeostasis in the treated hyperglycemic rats compared to the untreated hyperglycemic rats.

In this experiment the hypoglycemic activity of the fractions of *Dryopteris dilatata* could be attributed to the presence of the individual phytochemical that has been implicated in glucose homeostasis through several mechanisms of actions, Other previous reports from several plants studies have been reported to show potent blood glucose lowering effects on oral glucose loaded Wister rats [10, 23].

Observed reduction of body weight in alloxan-induced diabetic rats in present study is known to be associated with diabetes mellitus due to destruction of pancreatic beta cell and subsequent reduction of insulin. It may be as a result of muscle wasting, breakdown of complex molecules like carbohydrates, fats and proteins [24]. Treatment with fractions of *Dd* showed statistically significant increase in body weight compared to diabetic control revealing the therapeutic

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activity of *Dd* in muscle wasting and breakdown of complex molecules, modulating the activity of oxidative stress and gluconeogenesis observed in diabetic state [25].

In diabetes uncontrolled hyperglycemia is attributed to increased levels of serum TC, TG, VLDL, and LDL and subsequent reduction in the levels of serum HDL in diabetic non-treated rats. Significant alterations in the levels of lipid profile observed in current study correlates with observations from previous research on associated metabolic disorders in alloxan-induced diabetes [26]. After daily oral administration of fractions of *Dd* (800 mg/kg), abnormalities in lipid levels after diabetic induction was adequately reversed as levels of total cholesterol, tryglycerides, low density lipoproteins and very low density lipoproteins level were reduced and high density lipoprotein level was increased which could be associated to elevated levels of the hormone thyroxine that possess the capacity to remove circulating bad cholesterol back to the liver, showing the potency of fractions of *Dd* in controlling diabetes [27]. Our results correlate with Khan *et al*., [28] and KOFFI *et al*., [29] where methanolic extract of *Debregeasia salicifolia* and Cassia siame adequately managed diabetes and serum lipid profile in alloxan-induced diabetic rats.

Reports from researches observed the connection between diabetes mellitus and the generation of reactive species resulting from the action of alloxan on the pancreas and in hampered antioxidant defense showing the potential of lipid peroxidative activity in the development of hyperglycemic induced organ damaged [30]. Present study demonstrated elevated levels of MDA and reduced levels GSH, CAT and SOD in the brain and testis of diabetic non-treated rats and this was reversed in diabetic treated rats. CAT, SOD and GSH are potential endogenous antioxidants acting as internal defense mechanism protects against free radicals and prevent oxidative damage. Reduction in the levels of endogenous antioxidants in the brain and testis of diabetic control rats is the resultant effect of oxidative stress through the excessive production of reactive species, the action of lipid peroxidation and subsequently damage to organ in hyperglycemic state. In the present investigation induction of alloxan produced high levels of MDA and depleted levels of biomarkers of endogenous antioxidants in the brain an testis of diabetic control rats but 800 mg/kg of reducing sugar, alkaloid and tannin fractions of *Dd* reversed the increased levels of MDA and elevated the levels of GSH, SOD and CAT in the brain and testis of diabetic treated rats which align with records of earlier studies [31, 32].

Earlier reports evaluated the connection between hyperglycemia, generation of free radicals and depletion of antioxidant defense. Seen in our investigation is the reduction of the activities of SOD, GSH and CAT in the brain and testis of non-treated alloxan-induced diabetic wistar rats connoting the activity of excessive free radical production in diabetes. The mechanism of alloxan monohydrate in destroying the beta cells of the pancreas leading to absolute deficiency in insulin and finally diabetes promotes the lipid peroxidation [33]. In diabetic non-treated rats activity of oxidative stress marker was increased as hyperglycemic state enables continuous lipid peroxidation by enhancing the production of reactive species and maintained depletion of antioxidant markers, by autoxidation in glucose metabolism all of which accumulation leads to oxidative stress and cellular and organ damage observed in diabetic complication [34]. The major indicator of oxidative stress malondialdehyde (MDA) being highly reactive compound had elevated levels in diabetic non-treated rats which was brought to near normal in diabetic rats treated with fractions of *Dd* which could be as a results of the fractions capacity in regenerating the beta cells of the pancreas to produce insulin and creating sensitivity of the cells to insulin and mobilize glucose to be stored in the liver. The observed results agree with findings from Edo *et al*., [35] and Armenia *et al*., [36] where plant fractions reversed the activity of lipid peroxidation, restored destroyed insulin and reversed hyperglycemic state.

Conclusion

It is revealed in the present study that fractions of *Dryopteris dilatata* possess the potential to ameliorate hyperlglycemia, hyperlipidemia and oxidative stress as it reduces high glucose levels, serum lipid levels, levels of lipid peroxidation and increases endogenous and oxidants in alloxan-induced diabetic wistar rats.

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