




Research Article

Modulatory Effect of Fraction of *Dryopteris dilatata* on Alloxan-induced Oxidative Stress, Hepato-nephro Injury in Wistar Rat Models

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Abstract

The present study investigates the protective and modulatory effects of a bioactive fraction of *Dryopteris dilatata* against oxidative stress and hepato-renal damage induced by alloxan in Wistar rat models. Alloxan, a known diabetogenic agent, was administered to induce oxidative stress and subsequent hepatic and renal injury, simulating diabetic complications. Experimental rats were divided into control, alloxan-induced, and treatment groups receiving varying doses of the plant extract fraction. Biochemical parameters, including markers of oxidative stress (MDA, SOD, CAT, GPx), liver function (ALT, AST, ALP, bilirubin), and renal function (urea, creatinine, uric acid), were assessed. Histopathological evaluations of liver and kidney tissues were also conducted. The results demonstrated that treatment with the *Dryopteris dilatata* fraction significantly ameliorated oxidative stress, restored antioxidant enzyme activities, and improved liver and kidney function markers compared to the alloxan-only group. Histological observations further confirmed the protective effect, showing reduced cellular degeneration and improved tissue architecture. These findings suggest that the *Dryopteris dilatata* fraction exhibits potent antioxidant and organ-protective properties, making it a potential therapeutic candidate for managing oxidative stress-related hepatic and renal complications.

1. Introduction

Chronic metabolic disease such as diabetes mellitus (DM) has been a major concern to the global community as several complications emanates threatening health status and overall efficiency to individuals affected [1]. Morbidity and mortality rates in DM cases poorly managed is on the increase as the disorder results from inadequate insulin or its resistance to tissues leading to hyperglycemia and further increased reactive species and oxidative stress [2, 3]. Glucose transporters are glycoproteins that aids the uptake of glucose into cells,

GLUT-2 aids in regulating glucose metabolism in the liver, with metabolites which stimulates glucose sensitive transcription [4]. However, the major hormone responsible for regulating glucose metabolism is insulin and whenever the secretion uptake into cells is hampered results in an increase in blood glucose level [5, 6].

Alloxan is known to induce experimental diabetes as it damages the pancreatic beta-cells and with the aid of GLUT-2 are transported into plasma membrane of the beta-cells [7]. Complications in diabetes are resulted from alterations in normoglycemia and in glucose uptake which is linked to symptoms such as polyuria, polydipsia, polyphagia, weight loss, low wound healing and complications linked to cardiovascular disorders, neuropathy, nephropathy and many more [8, 9].

Several mechanisms have adopted in preventing, manage and treat hyperglycemia and its associated complications, moreover, its prevalence still presents a global burden, since time immemorial DM has been left without a cure, and present synthetic medicines for diabetes management such as insulin and the oral hypoglycemic together adequate nutrition with lifestyle changes still is insufficient [10, 11]. Also reports from previous studies have shown that synthetic remedies possess adverse reactions on prolonged use, so search for efficacious remedies with reduced or no toxic effect in the treatment, prevention and management of DM is important [12].

Traditional medicine therapy researchers have suggested that herbal remedies possess potential benefits in the management of several disorders including diabetes mellitus and complications attributed to it [13, 14]. Alkaloids, phenols, tannins, reducing sugar and many others are phyto-constituents found in plants having the potentials to combat various disease conditions through mitigating the damage caused by lipid peroxidation end product and regenerating endogenous antioxidants activities [15, 16]. Moreover, earlier studies have reported that plant rich in active compounds are able to regenerate damage done to the pancreatic beta cells in producing insulin and also improve the sensitivity of insulin to the muscle cells [17].

Dryopteris dilatata is one of the numerous plants used in ameliorating diabetes mellitus, it is a broad buckler fern in the family of Dryopterideaceae, dark in colour with green triinate fronds while brown scales cover it fibs [18]. It is locally called Okpomie in Isoko land in Nigeria and have been used traditionally for anti-dandruff, worm expeller [8, 11]. More so we have reported its anti-diabetic properties and associated complications in several context as a result of the bioactive components [11, 19]. In the present we evaluated the protective effect of fractions of *Dryopteris dilatata* on Alloxan induced oxidative stress, hepatonephro injury and histological alterations in wistar rat models.

2. Materials and Methods

2.1. Chemicals and Drugs

Santa-cruz (USA) provided the Alloxan monohydrate used, Metformin was gotten from a community pharmacy, Sigma Aldrich (Germany) produced Thiobabaturic acid (TBA), adrenaline and 5-Dithio-bis (2-nitrobenzoate) DTNB, Burgoyne Buidges and Co. (Mumbai, India) provided Trichloroacetic acid (TCA), Assay kits for renal and hepatic function test was purchased from Immunometrics Limited (UK). All other reagents and solvents were analytical grade.

2.2. Experimental Animals

Thirty-six adult male Wistar rats (135-140 g) were used for the study; they were purchased from the animal house of the Department of Pharmacology and Therapeutics, University of Nigeria, Enugu, Nigeria. The rats were kept under standard laboratory guidelines for animal care according to the University ethics which follows the Principle of Laboratory Animal Care (NIH Publication No.85-23). The rats were acclimatized for two weeks under normal laboratory condition (12 h light and dark cycles) allowing them free access food and water ad libitum.

2.3. Collection of Plant, Identification And Extraction

Dryopteris dilatata fresh leaves from a Olomoro community in Isoko South, Delta State, Nigeria, they were identified by Dr. Irehi of the Botany department of Delta State University, Nigeria and sent to the Forest Research Institute of Nigeria, Ibadan (FRIN) where a herbarium number was assigned, FHI 110338. Thereafter the leaves were washed, air dried at room temperature and grinded to fine powder preceding extraction. Thereafter, Akpotu et al., [18] plant crude extraction method was used. The grinded plant powder was soaked in 70% ethanol for 72 h and filtered with Whatman No. 2 filter paper, and the residue was further used crude extract for ethyl acetate following same procedure. After which each concentrated solvent was used for vacuum liquid chromatography were 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.

2.4. Induction of Experimental Diabetes

Diabetes was induced into rats after an overnight fast through a single intra-peritoneal injection of 100 mg/kg of Alloxan monohydrate dissolved in 0.9 M sodium chloride buffer of pH 7 according to the methods of [11]. Thereafter diabetic state was confirmed in the rats after 72 h of diabetes induction and rats with fasting blood glucose level above 200 mg/dl considered diabetic and used for the study.

2.5. Treatment Protocol

The rats were divided into six groups (n-6). Group 1 served as the normal control rats (non-diabetic rats) and received distilled water (5 ml/kg). Group 2-6 were induced with alloxan monohydrate and confirmed diabetic. Thereafter, Group 2 served as the diabetic control rats (negative control) which received distilled water (5 mg/kg). Group 3 received Metformin (50 mg/kg). Group 4 received 800 mg/kg reducing sugar fraction of *Dryopteris dilatata* (RSFDD). Group 5 received alkaloid fraction of *Dryopteris dilatata* (AFDD). And group 6 received tannin fraction of *Dryopteris dilatata* (TFDD) (80 mg/kg). All fractions of *Dryopteris dilatata* and metformin were administered daily using oral cannula, animals were monitored for signs of mortality and fasting blood glucose level taken at five days interval for 15 days with blood

gotten from the tail vein of the rats after an overnight fast using Accucheck Active glucometer (Roche diagnostic, Mannheim Germany).

2.6. Preparation of Blood Samples and Tissue Homogenates For Biochemical Analysis

At the end the experiment duration (15th day), the rats were euthanized through cervical dislocation. Blood collection was done through cardiac puncture and serum was obtained through centrifugation at 3,000 rpm using a bench top centrifuge (Bosch, UK). Organs such as the pancreas, kidney and liver were excised rinsed in phosphate buffer (0.1 M, pH 7.4), thereafter they were blotted with adsorbent paper. After that pancreas, liver and kidney were homogenized in sodium phosphate buffer (0.1 M pH 7.4) and cold centrifuged at 10,000 rpm for 10 minutes, the pancreas; liver and kidney were perfused insitu with 10 % formal saline and prepare for histomorphology examination. Tissue supernatant of pancreas, liver and kidney was collected and used for determination of catalase (CAT), reduced glutathione (GSH), superoxide dismutase (SOD) and malondialdehyde (MDA) activity. Kidney and liver biomarkers were also determined from the serum.

2.7. Determination of the Pro-Oxidant Activity (Lipid Peroxidation)

Oxidative stress activity in diabetic rats induced with alloxan was determined. The homogenates supernatant of the liver, kidney and pancreas were analyzed for lipid peroxidation end product biomarker malondialdehyde (MDA) following method of Adam-Vizi and Seregi [20]. Tissue concentration of thiobarbituric reactant assay (TBARS) expressed as gmol MDA/g tissue respectively.

2.8. Determination of The Activity of Endogenous Antioxidants (Catalase, Reduced Glutathione And Superoxide Dismutase)

The activity of catalase in the supernatants of pancreas, liver and kidney were analyzed following method of Goth et al., [21]. Following spectrophotometric protocol, the activity of CAT was estimated and the absorbance was read at a wavelength of 405 nm using UV/Vis spectrophotometer (INESA 750 N, china). The enzyme activity is expressed in kU/mg protein for all tissue respectively.

Levels of reduced glutathione in the tissue supernatant of the pancreas, liver and kidney were measured following methods of Sin et al., [22], using Elman reagent. The tissue supernatant was diluted to deproteinized it adding 1 ml of Trichloroacetic acid and centrifuged, after which the supernatant was mixed with 0.75 ml of sodium phosphate buffer (0.1 M, pH 7.4) together with 2 ml of 5, 5-Dithio-nitrobenzoic acid. The absorbance was read at 412 nm through a spectrophotometer (752N INESA, China) in less than 5 min and expressed in μM GSH/mL.

Levels of superoxide activity was measured following methods of Misra and Fridovich [23], based on the inhibition of adrenalin autoxidation in sodium carbonate buffer (pH 10.7). Autoxidation of adrenaline kinetics in the presence of tissue supernatant was observed for 3 min, increasing the absorbance and was of 495 nm and was observed at 60 and 240 seconds, and the activity expressed in U/mg protein for all tissues respectively.

2.9. Measurement of Liver Function Biomarkers

Alkaline phosphatase (ALP), Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) levels were measured in the serum using Randox test commercial kits (USA) according to manufacturer instructions [24].

2.10. Measurement of Renal Function Biomarkers (urea and creatinin)

Serum 50 ml was collected and mixed with a mono-reagent gotten from urea assay kit, after that the mixture was allowed to incubate for 60 sec and absorbance of 492 was use to read the mixture twice at interval of 1 min and the manufacturer instruction was used to calculate the concentration (Immunometrics Limited UK).

Serum urea was measured according to manufacturer's instruction using four parts of the mono-reagent from reagent 1 followed by one part of reagent 2 and incubated for 30 min at 15-35 C, after which the mixture kept before use. Thereafter 10 ml of serum sample and the standard were mixed up to 1,00 I/L mono-reagent for each and allowed to incubate for 50 sec at 20-30 C, and absorbance read at 340 nm two times at interval of 1 min and concentration calculated according to manufacturer's instruction (Immunometrics Limited UK).

2.11. Data Analysis

Data collected were expressed in mean \pm 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.

3. Results

3.1. Effect of fractions of *Dryopteris dilatata* on body weight of Alloxan-induced diabetic rats

After inducing the rats with Alloxan monohydrate there was statistically significant ($p < 0.05$) reduction in body weight in diabetic rats when compared to normal control rats Table 1, but treatment with fractions of *Dryopteris dilatata* (800 mg/kg) and Met. (50 mg/kg) caused a statistically significant ($p < 0.05$) elevation in the body weight compared to the diabetic control rats, though treatment with Met. (50 mg/kg) caused a statistically significant ($p < 0.05$) elevation in body when compared to RSFDD, AFDD and TFDD (800 mg/kg) treated rats Table 1.

Table 1: Effect of fractions of *Dryopteris dilatata* on body weight of Alloxan-induced diabetic rats. Data are expressed as means \pm 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

Table 1: Effect of fractions of *Dryopteris dilatata* on body weight of Alloxan-induced diabetic rats.

Groups	Initial Body weight (g)	Day 0 (g)	Day 5 (g)	Day 10 (g)	Day 15 (g)
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	130.07 ± 5.38 ^a	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 ^a	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 ^a	128.34 ± 4.80 ^b	129.58 ± 4.75 ^b	130.55 ± 4.72 ^{bc}
D + AFDD (800 mg/kg)	137.21 ± 3.49	124.57 ± 4.29 ^a	125.88 ± 4.16 ^b	127.58 ± 4.18 ^b	129.75 ± 4.05 ^{bc}
D + TFDD (800 mg/kg)	136.20 ± 5.28	127.10 ± 4.99 ^a	128.22 ± 4.87 ^b	129.29 ± 4.89 ^b	130.44 ± 4.84 ^{bc}

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*.

3.2. Fractions of *Dryopteris dilatata* decreases increased fasting blood glucose level in diabetic rats induced alloxan monohydrate

The analysis of fasting blood glucose levels in diabetic rats after alloxan induction were made at before alloxan induction, day 0, 5, 10 and day 15 as shown in Table 2 below.

After induction of alloxan there was statistically significant ($p < 0.05$) elevation in fasting blood glucose level in diabetic rats when compared to normal control at after alloxan induction, moreover at day 5, 10, and day 15, there was observed statistically significant ($p < 0.05$) increase in fasting blood glucose levels in the diabetic control rats when compared to the normal control rats, while rats treatment with FDD (800 mg/kg) and Met. (50 mg/kg) caused a statistically significant ($p < 0.05$) reduction in fasting blood glucose levels at day 5, 10 and day 15 when compared to diabetic control rats. Moreover, treatment with AFDD (800 mg/kg) at day 15 showed a statistically significant ($p < 0.05$) decrease in fasting blood glucose levels when compared to when compared to Met (50 mg/kg), RSFDD (800 mg/kg) and TFDD (800 mg/kg) treated rats as shown in Table 2.

Table 2: Effect of fractions of *Dryopteris dilatata* on body weight 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 ^{be}
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 ^{be}
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 ^{be}

Table 2: Effect of fractions of *Dryopteris dilatata* on fasting blood glucose level of Alloxan-induced diabetic rats. Data are expressed as means ± SEM (n=6), ^a $p < 0.05$ versus normal control, ^b $p < 0.05$ versus diabetic, 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*.

3.3. Fractions of *Dryopteris dilatata* attenuates markers of hepatic function in alloxan-induced diabetic rats

The activity of fractions of *Dryopteris dilatata* on biomarkers of hepatic function in rats induced with alloxan as shown in Table 3. There was observed statistically significant ($p < 0.05$) increase in serum ALP, AST and ALT following induction of alloxan in diabetic control rats when compared to the normal control rats, moreover administering FDD (800 mg/kg) and Met. (50 mg/kg) showed a statistically significant reduction in serum ALP, AST and ALT levels when compared to the diabetic control rats, while rats treated with AFDD (800 mg/kg) was observed with a statistically significant ($p < 0.05$) reduction in serum ALP, AST compared to RSFDD, TFDD (800 mg/kg) and Met. (50 mg/kg), but not significant ($p < 0.05$) against serum ALT when compared to Met. (50 mg/kg) treated rats Table 3.

Table 3: Effect of fractions of *D. dilatata* on liver function biomarker of diabetic rats.

Groups	ALP (U/L)	AST (U/L)	ALT (U/L)
Normal control	27.62 ± 4.35	36.42 ± 3.62	19.31 ± 1.23
Diabetic control	187.88 ± 21.85 ^a	136.82 ± 9.56 ^a	49.58 ± 1.77 ^a
D + Met 50 mg/kg	30.47 ± 2.55 ^{be}	38.71 ± 1.52 ^{be}	20.77 ± 73 ^{be}
D + RSFDD 800 mg/kg	32.47 ± 2. ^{be}	37.38 ± 1.53 ^{be}	25.24 ± 1.35 ^{be}
D + AFDD 800 mg/kg	29.99 ± 3.03 ^b	36.84 ± 1.61 ^b	20.56 ± 2.10 ^b
D +TFDD 800 mg/kg	34.34 ± 3.49 ^{be}	38.63 ± 2.67 ^{be}	23.92 ± 1.81 ^{be}

Table 3 Effect of fractions of *D. dilatata* on liver function biomarker of diabetic rats. Data are expressed as means ± SEM (n=6), $ap < 0.05$ versus normal control, $bp < 0.05$ versus diabetic, 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*.

3.4. Fractions of *Dryopteris dilatata* ameliorates biomarkers of renal function in alloxan-induced diabetic rats

Table 4 revealed the activity of fractions of *Dryopteris dilatata* in biomarkers of renal function in alloxan-induced diabetic rats.

There was observed statistically significant ($p < 0.05$) elevation in the levels of serum renal urea and creatinine in diabetic control rats following alloxan monohydrate induction compared to the normal control rats, however administering FDD (800 mg/kg) and Met. (50 mg/kg) showed a statistically significant ($p < 0.05$) reduction in serum urea and creatinine levels compared to diabetic control rats. More so diabetic rats treated with AFDD (800 mg/kg) showed a statistically significant ($p < 0.05$) decrease in serum urea and creatinin levels compared to RSFDD, TFDD (800 mg/kg) and Met. (50 mg/kg) treated rats Table 4.

Table 4: Effect of fractions of *D. dilatata* on renal function biomarkers of Alloxan-induced diabetic rats.

Groups	Creatinine (mg/dl)	Urea (mg/dl)
Normal control	0.59 ± .087	28.94 ± 1.36
Diabetic control	1.67 ± 0.05 ^a	58.67 ± 2.12 ^a
D + Met 50 mg/kg	0.96 ± 0.15 ^b	34.08 ± 1.33 ^b
D + RSFDD 800 mg/kg	1.46 ± 0.18 ^b	37.48 ± 1.84 ^b
D + AFDD 800 mg/kg	0.90 ± 0.18 ^b	30.44 ± 2.68 ^b
D +TFDD 800 mg/kg	1.35 ± 0.10 ^b	35.34 ± 1.56 ^b

Table 4 Effect of fractions of *D. dilatata* on renal function biomarkers of Alloxan-induced diabetic rats. Data are expressed as means ± SEM (n=6), ap<0.05 versus normal control, bp<0.05 versus diabetic, 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*.

3.5. *dryopteris dilatata* ameliorates lipid peroxidative activity in alloxan-induced diabetic rats

Table 5 above shows the levels of lipid peroxidative activity (MDA) in the pancreas, liver and kidney of alloxan induced-induced diabetic rats. There was observed statistically significant ($p < 0.05$) elevation in MDA levels in the pancreas, liver and kidney in the diabetic control rats when compared to the normal control rats, moreover treatment with FDD (800 mg/kg) and Met (50 mg/kg) showed a statistical significant ($p < 0.05$) reduction in the levels of MDA activity in the pancreas and liver and kidney compared to the diabetic control rats. Treatment with Met (50 mg/kg) caused a statistical significant ($p < 0.05$) reduction in the levels of MDA in the pancreas compared to RSFDD, AFDD and TFDD (800 mg/kg), also treatment with RSFDD (800 mg/kg) showed a statistical significant ($p < 0.05$) decrease of MDA activity in the liver compared to AFDD, TFDD (800 mg/kg) and Met (50 mg/kg), while treatment with AFDD (800 mg/kg) was observed with a statistical significant ($p < 0.05$) reduction of MDA activity in the kidney when compared to RSFDD, TFDD (800 mg/kg) and Met (50 mg/kg) Table 5.

Table 5: Effect of fractions of *D. dilatata* on renal function biomarkers of Alloxan-induced diabetic rats.

Groups	Pancreas	Liver	Kidney
Normal control	1.38 ± 0.11	1.12 ± 0.11	0.67 ± 0.16
Diabetic control	2.67 ± 0.10 ^a	2.40 ± 0.19 ^a	2.10 ± 0.28 ^a
D + Met (50 mg/kg)	1.65 ± 0.07 ^b	1.54 ± 0.12 ^{bd}	0.67 ± 0.06 ^{be}
D + RSFDD (800 mg/kg)	1.96 ± 0.20 ^{bc}	1.10 ± 0.14 ^b	1.05 ± 0.21 ^{be}
D + AFDD (800 mg/kg)	1.79 ± 0.24 ^{bc}	1.27 ± 0.21 ^{bd}	0.61 ± 0.09 ^b
D + TFDD (800 mg/kg)	1.96 ± 0.26 ^{bc}	1.53 ± 0.10 ^{bd}	0.72 ± 0.05 ^{be}

Table 5 Effect of fractions of *D. dilatata* on lipid peroxidation (MDA) (nM/mg protein) of Alloxan-induced diabetic rats. Data are expressed as means ± SEM (n=6), ap<0.05 versus normal control, bp<0.05 versus diabetic, 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*.

3.6. Fractions of *Dryopteris dilatata* rejuvenates endogenous anti-oxidants levels in alloxan-induced diabetic rats

Endogenous anti-oxidants levels (superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH) in alloxan-induced diabetic rats as shown in Table 6, 7 and 8.

The diabetic untreated rats (diabetic control) were observed with a statistically significant ($p < 0.05$) decrease in the activity of CAT, SOD and GSH in organs such as the pancreas, liver and kidney when compared to the normal control rats, while treatment with FDD (800 mg/kg) and Met. (50 mg/kg) caused a statistically significant ($p < 0.05$) elevation in the activity of CAT in the liver, kidney and pancreas in comparison to the diabetic control rats Table 6.

Table 6: Effect of fractions of *D. dilatata* on catalase (CAT) (nM/mg protein) level of Alloxan-induced diabetic rats. Data are expressed as means ± SEM (n=6), ^ap<0.05 versus normal control, ^bp<0.05 versus diabetic, 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*.

More so diabetic control rat was observed with a statistical significant ($p < 0.05$) reduction in the levels of SOD activity in the liver, kidney and pancreas when compared with the normal control rats, but treatment with FDD (800 mg/kg) and Met (50 mg/kg) showed a statistical significant ($p < 0.05$) increase in the levels of SOD activity in the pancreas, liver and kidney when compared with the diabetic control rats, however treatment with AFDD (800 mg/kg) was observed with a statistical significant ($p < 0.05$) increase in the activity of SOD

Table 6: Effect of fractions of *D. dilatata* on renal function biomarkers of Alloxan-induced diabetic rats.

Groups	Pancreas	Liver	Kidney
Normal control	31.41 ± 5.84	68.93 ± 5.82	67.94 ± 3.55
Diabetic control	20.22 ± 3.03 ^a	50.99 ± 8.88 ^a	28.25 ± 3.68 ^a
D + Met (50 mg/kg)	57.03 ± 2.57 ^b	66.95 ± 3.00 ^b	65.65 ± 2.32 ^b
D + RSFDD (800 mg/kg)	42.58 ± 2.89 ^b	86.68 ± 4.41 ^b	46.74 ± 2.91 ^b
D + AFDD (800 mg/kg)	53.33 ± 3.09 ^b	90.92 ± 7.79 ^b	67.41 ± 1.99 ^b
D + TFDD (800 mg/kg)	55.39 ± 2.53 ^b	59.11 ± 6.69 ^b	53.74 ± 4.41 ^b

in the pancreas, liver and kidney compared with RSFDD and TFDD (800 mg/kg), but not significant ($p < 0.05$) in the kidney to Met (50 mg/kg) treated rats Table 7.

Table 7: Effect of fractions of *D dilatata* on superoxide dismutase (SOD) (nM/mg protein) level of Alloxan-induced diabetic rats.

Groups	Pancreas	Liver	Kidney
Normal control	20.17 ± 0.94	35.34 ± 1.77	25.02 ± 1.55
Diabetic control	7.98 ± .65 ^a	21.72 ± 1. ^a	13.42 ± 0.81 ^a
D + Met (50 mg/kg)	14.48 ± 1.02 ^{be}	31.75 ± 1.79 ^{be}	22.19 ± 0.90 ^b
D + RSFDD (800 mg/kg)	11.64 ± 1.20 ^{be}	26.66 ± 0.94 ^{be}	16.33 ± 0.73 ^{be}
D + AFDD (800 mg/kg)	15.56 ± 0.77 ^b	34.40 ± 1.01 ^{be}	23.52 ± 0.83 ^b
D + TFDD (800 mg/kg)	12.84 ± 0.52 ^{be}	26.05 ± 2.01 ^{be}	15.44 ± 1.43 ^{be}

Table 7 Effect of fractions of *D dilatata* on superoxide dismutase (SOD) (nM/mg protein) level of Alloxan-induced diabetic rats. Data are expressed as means ± SEM (n=6), ^a $p < 0.05$ versus normal control, ^b $p < 0.05$ versus diabetic, 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 GSH in the diabetic control rats was seen to show a statistically significant ($p < 0.05$) reduction in the levels of GSH activity when compared to the normal control rats, while treatment with FDD (800 mg/kg) and Met (050 mg/kg) caused a significant ($p < 0.05$) elevation in the activity of GSH compared to the diabetic control rats. Moreover the was observed statistical significant increase ($p < 0.05$) in the levels of GSH activity in the liver in diabetic rats treated with AFDD (800 mg/kg) when compared with RSFDD, TFDD (800 mg/kg) and Met (50 mg/kg), while in the pancreas and kidney AFDD (800 mg/kg) also showed statistical significant ($p < 0.05$) increase in GSH activity but significant ($p < 0.05$) to the diabetic rat treated with Met (50 mg/kg) Table 8.

Table 8: Effect of fractions of *D. dilatata* on reduced glutathione (GSH) (nM/mg protein) level of Alloxan-induced diabetic rats.

Groups	Pancreas	Liver	Kidney
Normal control	51.42 ± .76	88.58 ± 7.02	71.37 ± 4.09
Diabetic control	30.28 ± 2.19 ^a	37.80 ± 4.09 ^a	38.80 ± 2.08 ^a
D + Met (50 mg/kg)	53.07 ± 3.77 ^b	57.04 ± 11.64 ^{be}	67.84 ± 1.32 ^b
D + RSFDD (800 mg/kg)	42.60 ± 2.15 ^{bce}	50.36 ± 10.29 ^{be}	58.56 ± 2.86 ^{be}
D + AFDD (800 mg/kg)	51.59 ± 2.37 ^b	72.03 ± 1.72 ^b	67.29 ± 2.73 ^b
D + TFDD (800 mg/kg)	42.25 ± 2.42 ^{bce}	56.47 ± 2.26 ^{be}	59.44 ± 2.11 ^{be}

Table 8 Effect of fractions of *D. dilatata* on reduced glutathione (GSH) (nM/mg protein) level of Alloxan-induced diabetic rats. Data are expressed as means ± SEM (n=6), ^a $p < 0.05$ versus normal control, ^b $p < 0.05$ versus diabetic, 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 dilatate*.

4. Discussion

Previous research outlined prolonged hyperglycemia as a base for developing harmful effects in the human body in organs such as the pancreas, liver kidney cardiovascular system and the brain, this could be attributed to over generation of reactive oxygen and nitrogen species which alters the morphology of genes, causing mutations of these genes and thereafter producing unwanted proteins [25, 26]. Furthermore, retrospective studies reveal active components in plants such as alkaloids, flavonoids, phenols and many more that ameliorates derangements in metabolism and other complications in animal models [27].

In our investigation we obtained results on fractions of *Dryopteris dilatata* (FDD) that are same with retrospective results from extenuative capacity of other traditional medicinal plants on diabetes and associated complications [28, 29]. Results gotten from our investigation revealed potencies which indicates that using *Dryopteris dilatata* for treatment has the potential to ameliorate diabetes and attributed complications. Results from our previous study reveals the presence several phytochemical constituents contained in Dd [11, 30] with the ability to rejuvenate disease conditions, and promote health status through improving the body defence [11, 18]. Phenolic and polyphenolic compounds possess free radicals scavenging capacity, making them antioxidants [31]. Several herbal plants have been reported with connecting link between the content of phenols and antioxidant potentials [32].

Moreover, treatment with FDD regenerated the damage of alloxan-induced hyperglycemia. The ability of continuous administration of FDD to significantly decrease fasting blood glucose level in alloxan-induced diabetes was evident; however, this was not achieved on a single dose of administration of FDD. Our results also recorded hyperglycemic to near normal in diabetic rats treated with FDD at the interval of

glucose check (day 5, 10 and day 15) when compared to the initial glucose level. In the treatment of diabetes ameliorating hyperglycemia to near normal is the main aim that must be achieved to prevent the complications that are associated with diabetes [33, 34].

Poor glycaemic control has been shown to be the major contributing factor to developing complications in diabetes which includes macro and microvascular complications, a lot of plant remedies with active compounds have been shown with the potentials to normalize glycaemic levels in diabetes [18, 35]. Therefore our reports from this investigation imply that *Dryopteris dilatata* could be of great importance in diabetic condition to control blood glucose level.

Rapid production of free radicals leading to oxidative stress in hyperglycemic state has been implicated in the pathophysiology diabetic related disorders [26]. Generation of reactive oxygen and nitrogen species in prolonged hyperglycemia cause oxido-inflammation results in organs and tissue stress in diabetic condition, this is correlated to the imbalance between free radicals generation and endogenous antioxidants in favor of free radicals [36, 37], which have the tendency cause damage right from the cellular level to the tissues as such cause injury to the cells and tissues to the complications seen in diabetes [38]. Alloxan-induced diabetes in wistar rats caused significantly high levels of oxidative damage in the pancreas, liver and kidney of the diabetic control rats.

Cellular lipid peroxidation is a product of the interruption of oxidative and antioxidative system process [39], inability of the cell to eradicate toxic substance due to over production of free radicals can lead to organ failure [40]. In the present study, FDD significantly decreased the high levels of malondialdehyde in the pancreas, liver and kidney of diabetic treated rats. Antioxidants biomarkers activities such as catalase, reduced glutathione, and superoxide dismutase was observed to significantly decreased in the pancreas, liver and kidney of rats induced with alloxan in our investigation which an indication of increased oxidant generated by reactive species. Endogenous antioxidants like GSH, CAT and SOD are implicated in promoting defense mechanism by acting to scavenge and detoxify the system of free radicals [41].

Low levels of the activity of endogenous antioxidants observed in the diabetic control rats may be due to increased activity of lipid peroxidation in hyperglycemic state [42], leading to the elevated activity of malondialdehyde in the pancreas, liver and kidney due to over expression of O₂⁻ and H₂O. However, treatment with FDD ameliorated the low reduced activity of GSH, SOD and CAT in the pancreas, liver and kidney of alloxan-induced diabetic rats, implying that the plant fractions possess the capacity to scavenge free radicals.

The organ injury caused by alteration in metabolic activities induced by alloxan in diabetic rats on hepato-nephro markers were ameliorated in rats treated with FDD. Rats exposed to alloxan induction revealed deleterious injury to the liver and kidney as seen in the elevated levels of Alanine amino transferase (ALT), Aspartate Aminotransferase (AST) and Alkaline Phosphatase (ALP) activities, more so there was increased levels of urea and creatinin. Moreover, repeated administration of FDD reversed the biomarkers of liver and kidney in the serum of diabetic treated rats. Kidney and liver damage are complications that are always seen in diabetic condition [43].

Uncontrolled gluconeogenesis and elevated protein concentrations which are implicated in renal and liver injury induced by diabetes. Increased levels of liver enzymes are attributed to conversion of amino acids to keto acids [44, 45]. The protective activity of the liver and the kidney displayed by the fractions of *Dryopteris dilatata* reported in this study might be linked to its free radical scavenging ability implicated in the reduction of the levels of malondialdehyde and increased SOD, GSH and CAT levels. However, the ability of fractions of *Dryopteris dilatata* to scavenge free radicals in rats induced with diabetes could suggest that the plant fraction has potent antioxidant defense mechanism. Having recorded this activity, the plant *Dryopteris dolatata* has the capability to protect against hyperglycemia hepatic and renal damage in diabetic state.

5. Conclusion

Conclusively, the present investigation revealed the therapeutic capacities of *Dyopteris dilatata* towards glucose imbalance, also the anti-diabetic and protective effect against hepatic and renal dysfunction is in correlation with its antioxidant potencies which could have resulted from the phyto-active components in leaf.

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