Original Research Article

Methanol extract of *Dryopteris dilatata* alleviates streptozotocin-induced hyperglycaemia and liver injury in male Wistar rats.

Abstract

Dysfunctional pancreatic beta cell and impaired glucose metabolism in the liver has been implicated in streptozotocin (STZ)-induced diabetes mellitus and this has been linked with increased oxidative stress. Whether methanol extract of *Dryopteris dilatata* (MEDd), a flavonoid-rich plant can ameliorate STZ-induced liver damage remains an issue. Hence, this study investigated the effect of methanol extract of *Dryopteris dilatata* on STZ-induced diabetes mellitus in male Wistar rat. Animals were randomly selected into five groups (n=5) and were treated as follows; group 1 received distilled water (10ml/kg), Group 2 received only STZ (60mg/kg), Group 3 and 4 received STZ then 400 and 800mg/kg of MEDd respectively while Group 5 received STZ then pioglitazone (10mg/kg). Following 14 days of treatment, animals were euthanized and blood as well as spleen, liver, pancreas and kidney tissues were collected for further studies. Our results revealed that MEDd significantly reduced STZ-induced hyperglycaemia in Diabetic rats. Markers of oxidative injury (MDA, NO and GSH) were also significantly ameliorated in the pancreas and liver of the diabetic rats following treatment with MEDd. However, liver injury markers (ALT, AST and ALP) were significantly attenuated with marked decreased in organ weight in the diabetic rats after treatment with MEDd. We found that methanol extract of *Dryopteris dilatata* demonstrated anti-diabetogenic and hepato-protective potential by enhancing in vivo hepato-pancreatic antioxidant defense system.

Keywords: *Dryopteris dilatata*, Streptozotocin, Diabetes mellitus, Antioxidants, Liver injury

Introduction

Diabetes mellitus (DM) is one of the most well-known chronic metabolic illnesses. It has been reported that it poses a hazard to human health and has had a significant negative impact in the twenty-first century. According to predictions, DM cases will continue to rise globally, perhaps leading to an increase in mortality rates if not properly controlled [1, 2]. This

metabolic disease is caused by the pancreas inability to make insulin (insulin deficit) or properly use the insulin released (insulin resistance), or both, which can be caused by oxidative and inflammatory stress, among other things
[3, 4, 5]. However, this condition resulted in elevated blood sugar levels, which are common in diabetic patients. The glycoproteins family, which includes glucose transporters (GLUTs), is responsible for glucose uptake in the cell (Simmons 2017). GLUT-2 regulates the liver's glucose transporter, which supports glucose metabolism while also supplying molecules that promote the transcription of glucose-sensitive genes [6, 7]. Furthermore, insulin is responsible for glucose metabolism regulation and blocking insulin production resulted in increased hepatic glucose release and decreased glucose absorption in muscle cells [8, 7]. Chemically active diabetogenic drugs like streptozotocin have been employed in laboratory animal models to study type 1 diabetes. The GLUT-2 glucose uniporter transports this drug through the plasma membrane of pancreatic beta-cells, specifically impairing them [9, 10, 11, 12]. Hyperglycemia, hyperlipidaemia, hypertension, nephropathy, neuropathy, polyuria, polyphagia, stroke, ketosis, and other diabetic complications resulted from changes in glucose uptake [13, 14]. There have been numerous advancements in the prevention, treatment, and management of glycemic changes that contribute to DM. However, the incidence of DM-related complications is still a major concern around the world [15]. The preferred anti-diabetic drugs used in its management or treatments has been reported to cause adverse reactions in the body after administration; therefore, finding a low-toxic and effective functional food product or less-adversely reactive drug for the prevention and management of DM is extremely important.

Nutraceuticals or medicinal plants, according to ethnopharmacology and therapeutic studies, may help manage diabetes and its consequences [16, 17, 18, 20]. Phytochemicals found in various plant-based components, such as vitamins, polyphenols, tannins, flavonoids, saponins, terpenes, and sterols, can boost the body's antioxidant defence mechanism [21, 18]. However, a large number of studies have suggested that phyto-bioactive compounds with high antioxidant activity may have a variety of biological effects, including influencing glucose absorption and uptake, modulating insulin secretion, and managing or reducing the risk of developing DM complications [4, 14, 22]. *Dryopteris dilatata* is one of the plants used to treat health problems and the *Dryopteris dilatata*, popularly known as the 'Broad buckler fern,' is a member of the Dryopteridaceae family. It has dark green tripinnate fronds with brown scales on the ribs, and its ribs are covered in brown scales (Runk *et al.,* 2012). It's known as Okpomie and is found primarily in Nigeria's tropical region (Mordi *et al.,* 2016;

Akpotu *et al.*, 2018). The roots and leaves of the plant have a variety of medical uses, including treating dandruff on the scalp and removing worms from the body [23]. We also discovered that an ethanol extract of the plant's leaves has anti-hyperlipidemic properties [24]. Our research also demonstrated that plant leaves contain a large number of phytochemicals and active substances [24]. The effect of a methanol extract of *Dryopteris dilatata* (MEDd) on type 1 diabetes after streptozotocin administration was studied in this study using a mechanism involving endogenous antioxidant activities and liver function indicators.

Materials and Procedures

Chemicals and drugs

Santa-cruz provided the streptozotocin (USA). In Port-Harcourt, Nigeria, pioglitazone was acquired at a community pharmacy. Sigma Aldrich produced thiobarbituric acid (TBA), Griess, and Ellman reagents (Germany). Burgoyne Burbidges & Co. (Mumbai, India) provided the trichloroacetic acid (TCA), and Immunometrics Limited provided the insulin ELISA kit, AST, ALT, and ALP kits (UK). The rest of the reagents and solvents were of analytical quality.

Source of Experimental Animals

Twenty-five mature male Wistar rats weighing 120-150 g were purchased from the Department of Pharmacology and Therapeutics, PAMO University of Medical Sciences, Port Harcourt, Nigeria's central animal house. The animals were kept in regular laboratory conditions, as per the University's ethical guidelines, which adhere to the "Principle of Laboratory Animal Care" (NIH Publication No. 85-23). The rats were given free access to the regular rat meal (Ladokun feeds) and water for one week (12–12 h light–dark cycle, 28 2°C).

Experimental Design

The animals were divided into five groups of six animals in each group

Group 1 (Normal control)- Fed with normal feed and water ad libitum

Group 2 (Diabetic control)- Fed with normal feed after induction of diabetes

Group 3 (Treated group)- Fed with normal feed and water ad libitum after induction of diabetes with daily treatment with 800 mg/kg of Dd

Group 4 (Treated group)- Fed with normal feed and water ad libitum after induction of diabetes with daily treatment with 400 mg/kg of Dd

Group 5 (Treated group)- Fed with normal feed and water ad libitum after induction of diabetes with daily treatment with 500 mg/kg of pioglitazone

Plant Collection and Identification and Extract Preparation

Dryopteris dilatata fresh leaves were obtained from the Olomoro community in Isoko South, Delta State, Nigeria. The leaves were authenticated for herbarium numbering, FHI 110338, at the Forestry Research Institute of Nigeria (FRIN), Ibadan. Before extraction, the leaves were washed, air dried, and macerated into powder form. Furthermore, the Kemelayefa and Kagbo plant crude extraction methods were modified (2018). 70 percent methanol was used to extract the mixed powder, which was then filtered using Whatman No. 2 filter paper. A rotary evaporator was then used to vacuum concentrate the solvent to dryness. The extract was then dried at 40°C in an incubator before being kept at 0-4°C. The yield of the extract was 13%. Finally, the dried extract was diluted in normal saline to get the needed dose of 800 mg/kg.

Diabetic Induction in Rats

Fasted male Wistar rats were given a single intraperitoneal injection of streptozotocin (STZ; 60 mg/kg) in sterile citrate buffer (0.1 M, pH 4.5) to develop Type 1 Diabetes mellitus, as described by Asiwe *et al*., [25]. The rats' diabetic status was determined after 72 hours using a glucometer (ACCU-CHEK® Active) and appropriate blood glucose test strips, and animals with a fasting blood glucose level of more than or equal to 200 mg/dl were chosen for the study [25].

Preparation of Blood Samples and Tissue Homogenates For Biochemical Analysis

At the end of the treatment duration, the rats were subjected to deep ether anaesthesia before euthanasia through cervical dislocation. Cardiac puncture was used for blood collection; the plasma was obtained by centrifugation at 3,000 rpm at room temperature using a bench top centrifuge (Bosch, UK). The pancreas, spleen and liver were excised, rinsed in iced-cold Tris-KCl buffer (0.1 M, pH 7.4), and then blotted with adsorbent paper. Furthermore, the liver and pancreas were homogenized in sodium phosphate buffer (0.1 M, pH 7.4), centrifuged at 10,000 rpm for 10 min at 4 ^{0}C. For biochemical analysis, the tissue supernatant (pancreas and liver) was collected and used for the estimation of malondialdehyde (MDA),

Nitrite (NO) and reduced Glutathion (GSH). Liver function tests were also estimated from the plasma. For biochemical analysis, the tissue supernatant (pancreas and liver) was collected and used for the estimation of malondialdehyde (MDA), Nitrite (NO) and reduced Glutathion (GSH). Liver function tests were also estimated from the plasma.

Estimation of Levels of Lipid Peroxidation

The amount of oxidative stress in diabetic rats caused by streptozotocin was calculated. The lipid peroxidation end product marker malondialdehyde was measured in the pancreas and liver supernatants using the thiobarbituric reactive assay (TBARS) as previously described [26, 27]. The amounts of TBARS in the tissues were measured in gmol MDA/mg tissue protein.

Measurement of Tissue Nitrite

Spectrophotometric methods with Griess reagent were used to quantify nitrite in the pancreas and liver. Griess reagent was made in a 1:1 ratio from reagents A (1 percent sulfanilamide in 5% phosphoric acid) and B (0.1 percent N-1-naphthyl ethylenediamine dihydrochloride). Griess reagent was used to incubate the samples, which were then examined at 540 nm in a spectrophotometer. A standard curve of sodium nitrite (0–100 M) was used to estimate the nitrite content.

Estimation of Glutathione Reduction

The amount of reduced glutathione (GSH) in pancreas and liver supernatants was determined using Ellman's reagent in a modified approach [28]. 0.1 mL cell-free exudate supernatant was diluted 10 times and deproteninized with 1 mL Trichloroacetic acid (20%) before centrifugation at 10,000 rpm for 10 minutes at 4°C. The supernatant was then combined with 0.75 mL sodium phosphate buffer (0.1 M, pH 7.4) and 2 mL 51, 51-Dithios-nitrobenzoic acid (0.0006 M) (DTNB). In a UV/Vis spectrophotometer, the absorbance was measured at 412 nm in less than 5 minutes (752N INESA, China). The glutathione concentration, which was determined using a standard curve generated with standard glutathione (0–200 μM), was expressed as a function of the volume of tissue supernatant (μM GSH/mL of supernatant).

The Activity of The Liver Function Indicators

Alanine aminotransferase (ALT), Aspartate transaminase (AST), and Alkaline Phosphatase (ALP) for liver damage mediators in the plasma were evaluated using a Randox test kit, as described by Reitman and Frankel [29].

Serum Insulin Estimation

Following the manufacturer's instructions, the concentration of insulin in the blood was determined using an ELISA kit (Elabscience, UK).

Data Analysis

All data collected were represented as Mean ± SEM (standard Error of Mean) and analysed using one-way and two-way analysis of variance (ANOVA) followed by Bonferroni post hoc multiple comparison tests. $P<0.05$ was considered statistically significant. Graphs and statistical analyses were performed in GraphPad Prism software version 5.01 (GraphPad Software, Inc. La Jolla, CA 92037 USA).

Results

Change in body weight

There was no significant change in body weight of animals at week 0. However, at week 1 and 2, diabetes control animals showed a reduced body weight but following treatment with MEDd and pioglitazone at week 1 and 2, the body weight were significantly normalized as presented in figure 1.

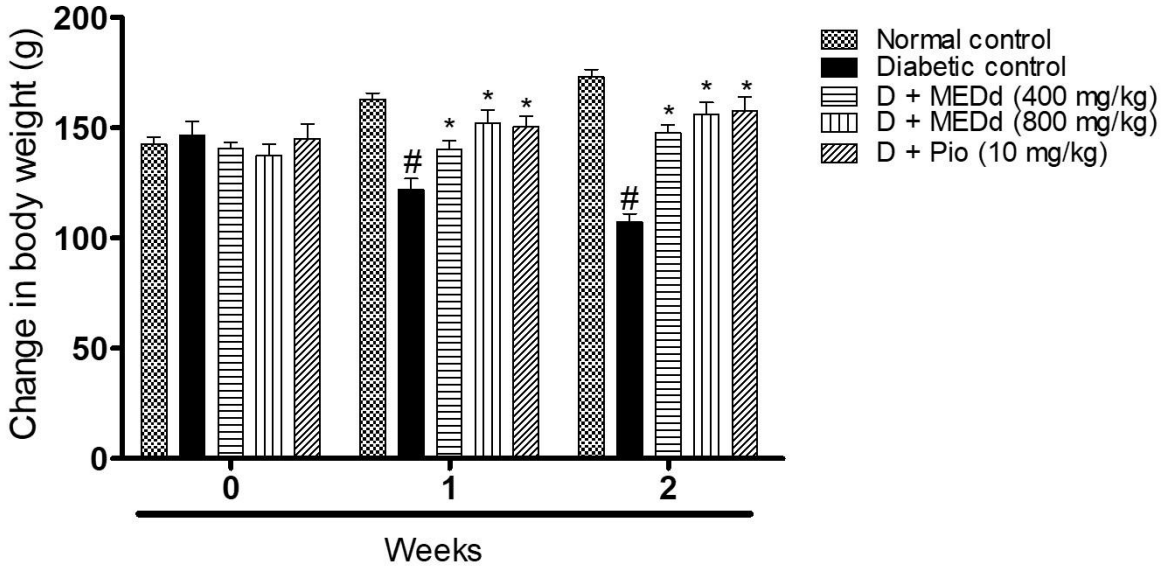

Fig. 1: change in body weight (g).

All values are expressed as Mean ± standard error of mean, (n=5), *p< 0.05 when compared with the diabetes control while $^{\#}p$< 0.05 was significant when compared with the normal control. D = streptozotocin induction, MEDd = methanol extract of *Dryopteris dilatata,* Pio = Pioglitazone.

Relative weight of Spleen, Liver and Kidney

Figure 2 shows that there was no significant difference in the weight of spleen across the experimental groups. However, the liver showed a marked increase in diabetes control group. Following treatment with MEDd (400 and 800mg/kg) and pioglitazone (10mg/kg), the liver weight was significantly reduced. Similar data was observed in the weight of kidney though MEDd (400mg/kg) did not have any significant effect on the weight of the kidney when compared with the control.

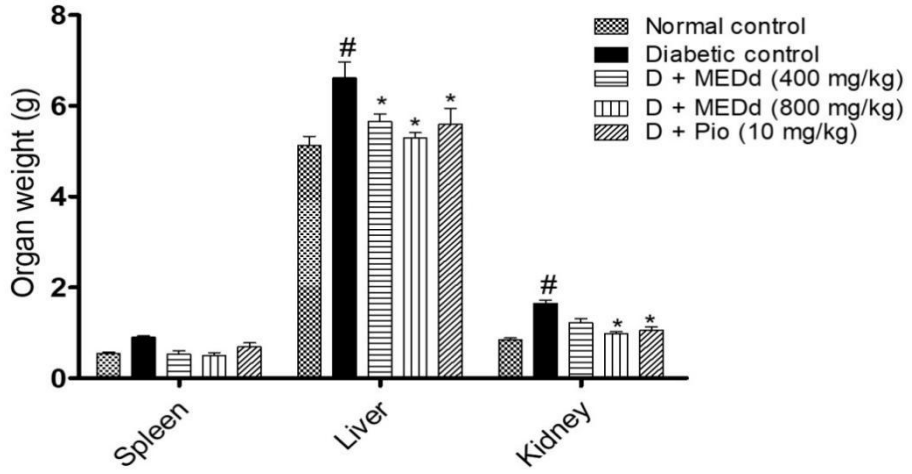

Fig. 2: organ weight of spleen, liver and kidney.

All values are expressed as Mean ± standard error of mean, (n=5), *p< 0.05 when compared with the diabetes control while $^{\#}p$< 0.05 was significant when compared with the normal control. D = streptozotocin induction, MEDd = methanol extract of *Dryopteris dilatata,* Pio = Pioglitazone.

Fasting blood Sugar

Following the induction of diabetes with STZ, the fasting glucose level was observed to be above 200mg/dl after 72hrs of induction. However, treatment with MEDd (400 and 800mg/kg) and pioglitazone significantly reduced the glucose level in a duration dependent manner as presented in figure 3.

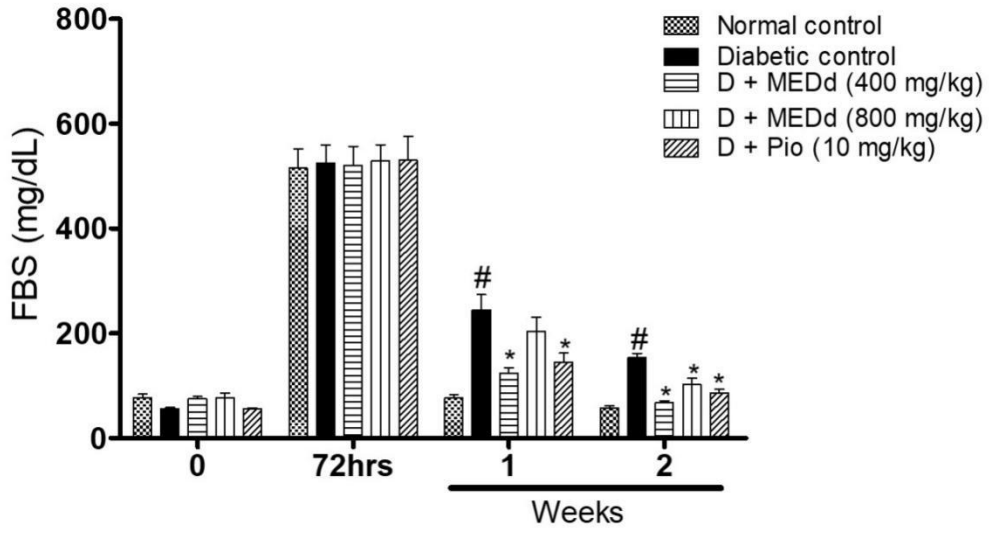

Fig. 3: Fasting Blood Sugar

All values are expressed as Mean ± standard error of mean, (n=5), *p< 0.05 when compared with the diabetes control while $^{\#}p$< 0.05 was significant when compared with the normal control. D = streptozotocin induction, MEDd = methanol extract of *Dryopteris dilatata*, Pio = Pioglitazone.

Glucose tolerance test

Following oral administration of glucose, the glucose level in diabetes control group was significantly increased in 30, 60, 90, 120 and 150 minutes when compared with the normal control. However, treatment with MEDd (400 and 800mg/dl) and pioglitazone (10mg/kg) significantly reduce the glucose level in 30, 60, 90, 120, and 150 minutes when compared with diabetes control but was significantly elevated when compared with normal control animals as shown in figure 4.

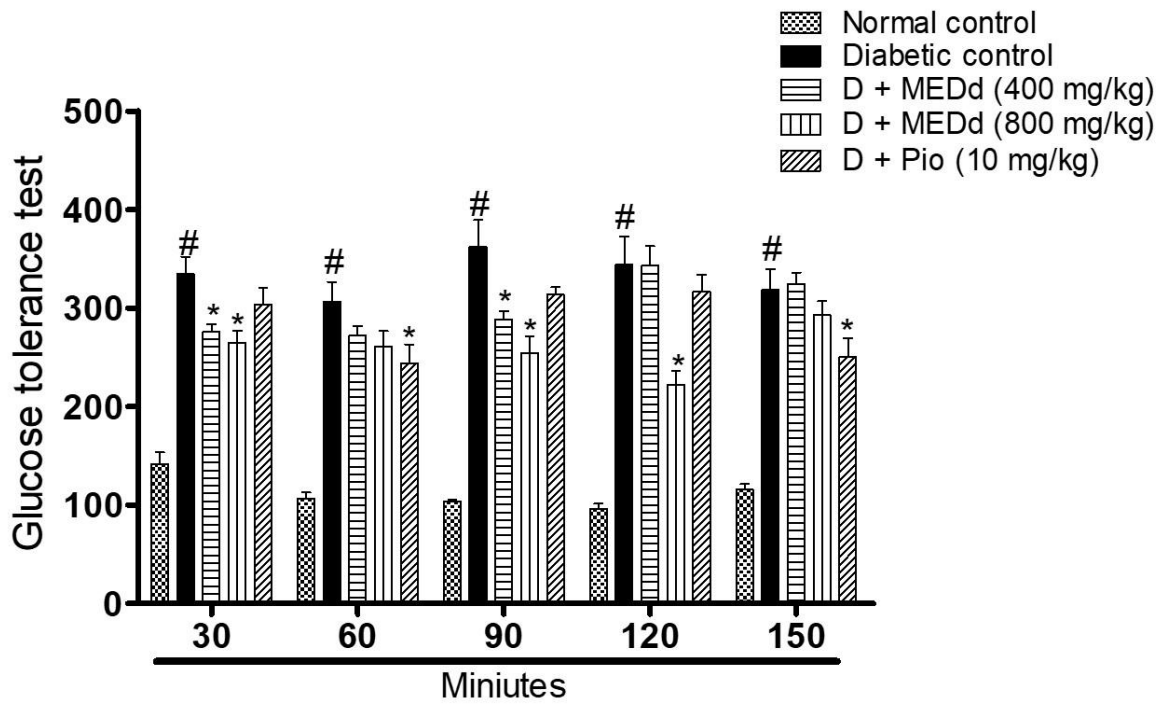

Fig. 4 Oral Glucose Tolerance (OGT)

All values are expressed as Mean ± standard error of mean, (n=5), *p< 0.05 when compared with the diabetes control while $^{\#}p$< 0.05 was significant when compared with the normal control. D = streptozotocin induction, MEDd = methanol extract of *Dryopteris dilatata,* Pio = Pioglitazone.

Serum insulin

Figure 5 represent the serum insulin concentration. The serum insulin concentration was significantly reduced in diabetes control animal following the STZ induction. However, treatment with MEDd (400 and 800mg/kg) and pioglitazone significantly increase the serum concentration of insulin

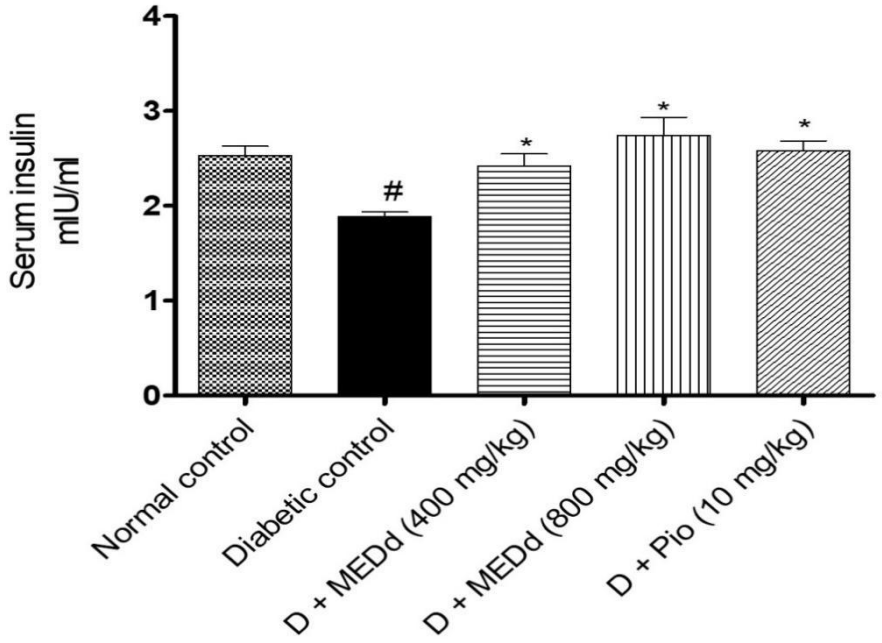

Fig. 5: Serum insulin concentration

All values are expressed as Mean ± standard error of mean, (n=5), *p< 0.05 when compared with the diabetes control while $^{\#}p$< 0.05 was significant when compared with the normal control. D = streptozotocin induction, MEDd = methanol extract of *Dryopteris dilatata,* Pio = Pioglitazone.

Liver enzymes

Figure 6-8 represents the markers of liver injury. Streptozotocin induction significantly increases serum ALP, AST and ALT in diabetes control rats when compared with normal control rats. However, the treatment of MEDd (400 and 800mg/dl) and pioglitazone significantly reduce AST and ALT when compared with diabetes control animals (fig 6 and fig. 7). ALP was not significant different when MEDd (400mg/kg) was compared with the diabetes control animals (fig. 8)

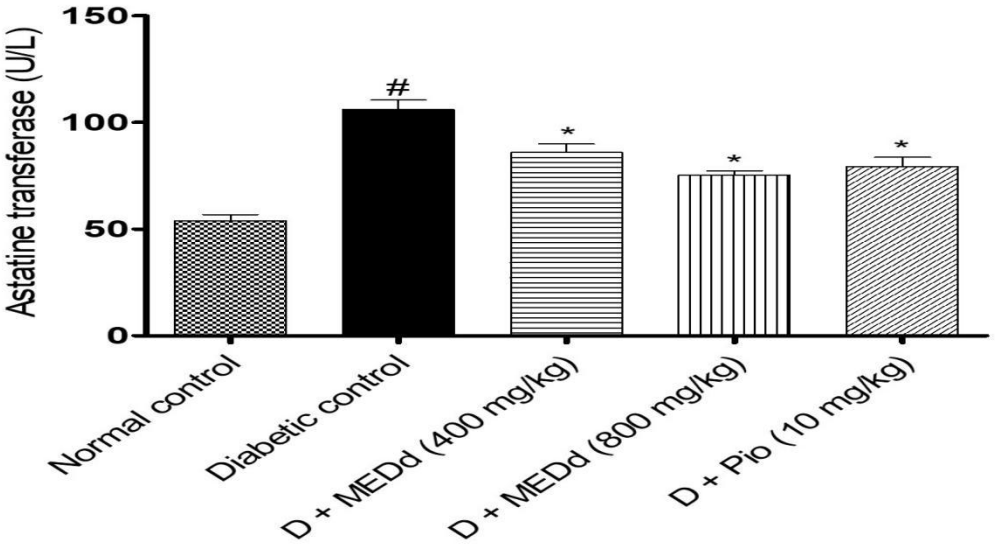

Fig. 6: Aspartate transaminase (AST)

All values are expressed as Mean ± standard error of mean, (n=5), *p< 0.05 when compared with the diabetes control while $^{\#}p$< 0.05 was significant when compared with the normal control. D = streptozotocin induction, MEDd = methanol extract of *Dryopteris dilatata,* Pio = Pioglitazone.

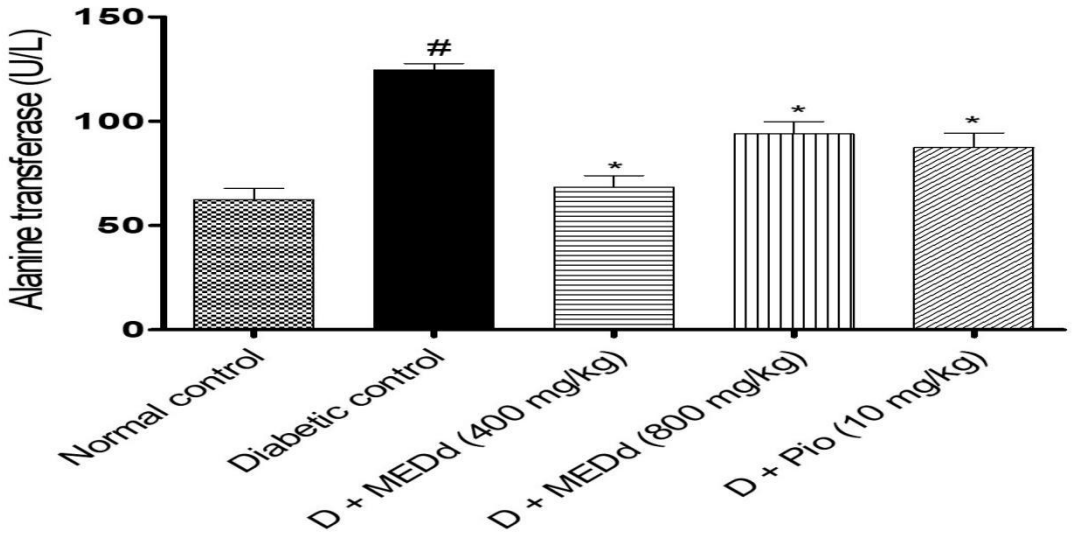

Fig 7: Alanine amino transferase (ALT)

All values are expressed as Mean ± standard error of mean, (n=5), *p< 0.05 when compared with the diabetes control while $^{\#}p$< 0.05 was significant when compared with the normal control. D = streptozotocin induction, MEDd = methanol extract of *Dryopteris dilatata,* Pio = Pioglitazone.

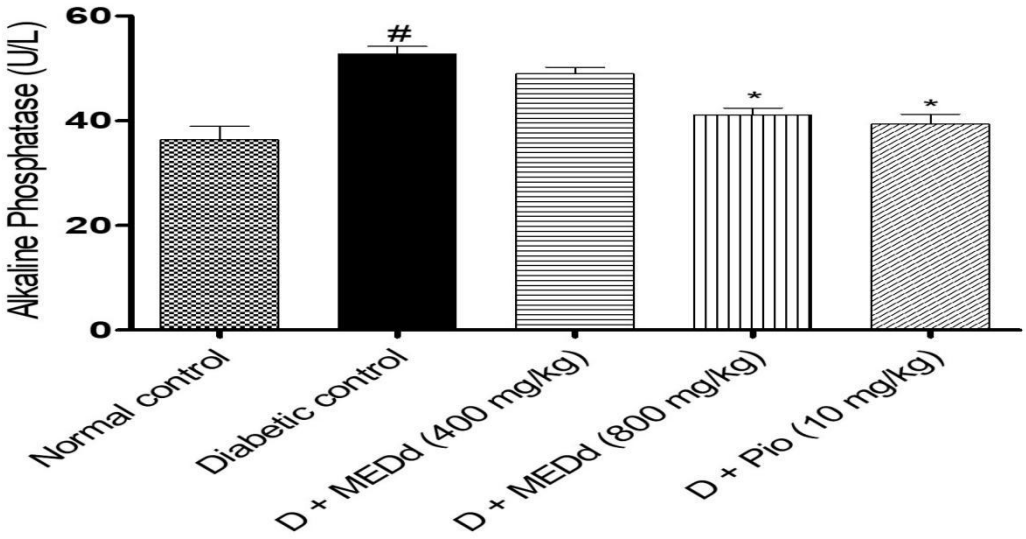

Fig. 8: Alkaline Phosphatase (ALP)

All values are expressed as Mean ± standard error of mean, (n=5), *p< 0.05 when compared with the diabetes control while $^{\#}p$< 0.05 was significant when compared with the normal control. D = streptozotocin induction, MEDd = methanol extract of *Dryopteris dilatata,* Pio = Pioglitazone.

Pancreatic antioxidants

Presented in figure 9-11 is the pancreatic oxidative stress marker. There was significant increase in pancreatic MDA and Nitrite level in diabetes control animals while GSH level was significantly reduced in diabetes control animal. However, following treatment with MEDd (400 and 800mg/dl) and pioglitazone (10mg/kg) significantly reduces the MDA as well as elevated the pancreatic GSH level when compared to diabetes control animal. Treatment with MEDd (400 and 800mg/kg) did not show any significant difference in pancreatic Nitrite level when compared with diabetes control animal.

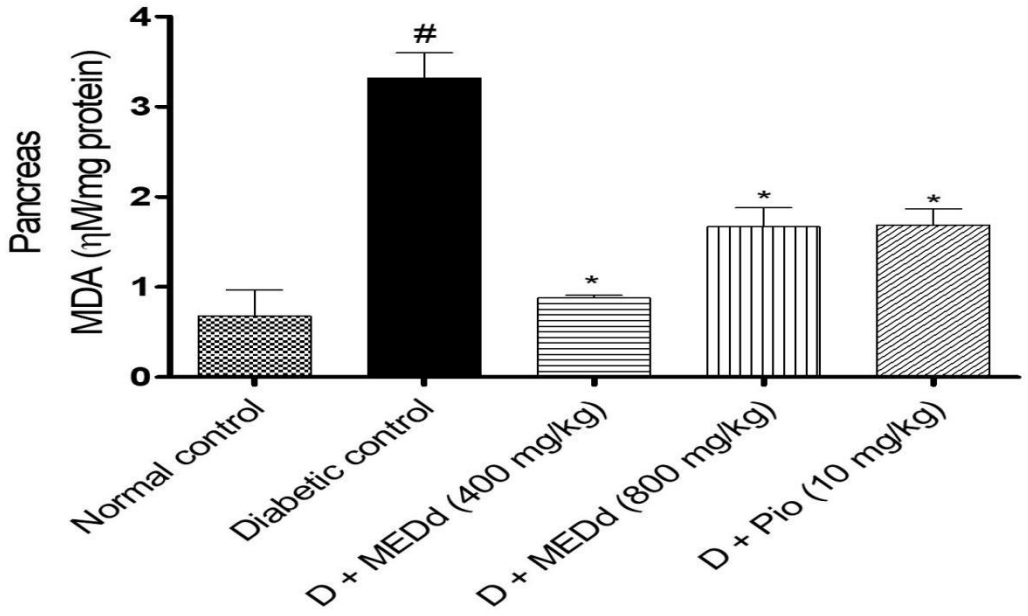

Fig. 9: Pancreatic MDA level.

All values are expressed as Mean ± standard error of mean, (n=5), *p< 0.05 when compared with the diabetes control while $^{\#}p$< 0.05 was significant when compared with the normal control. D = streptozotocin induction, MEDd = methanol extract of *Dryopteris dilatata,* Pio = Pioglitazone.

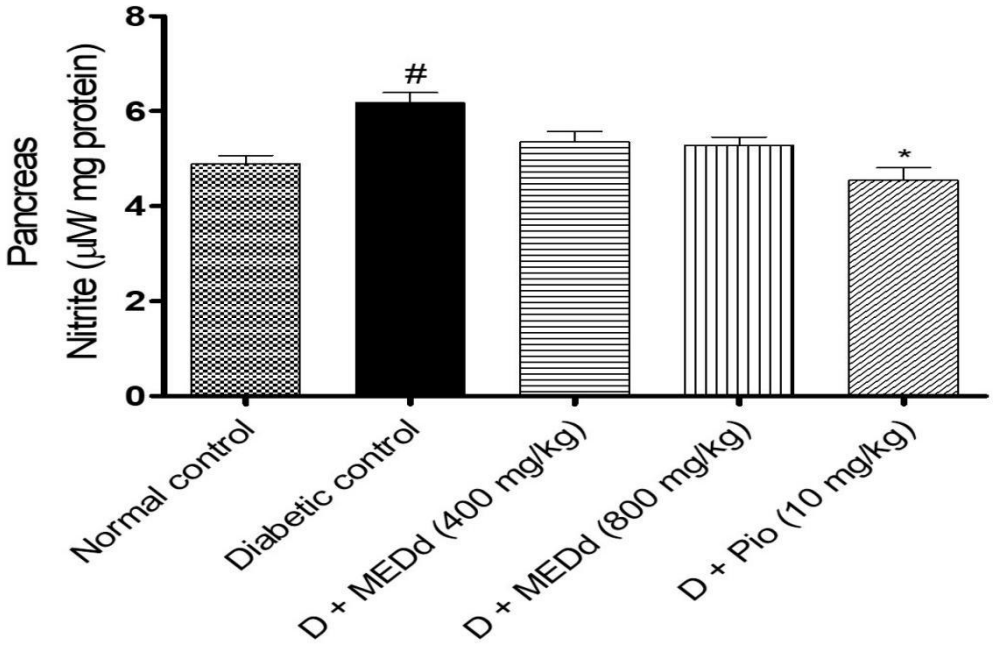

Fig.10: Pancreatic Nitrite level

All values are expressed as Mean ± standard error of mean, (n=5), *p< 0.05 when compared with the diabetes control while $^{\#}p$< 0.05 was significant when compared with the normal control. D = streptozotocin induction, MEDd = methanol extract of *Dryopteris dilatata,* Pio = Pioglitazone.

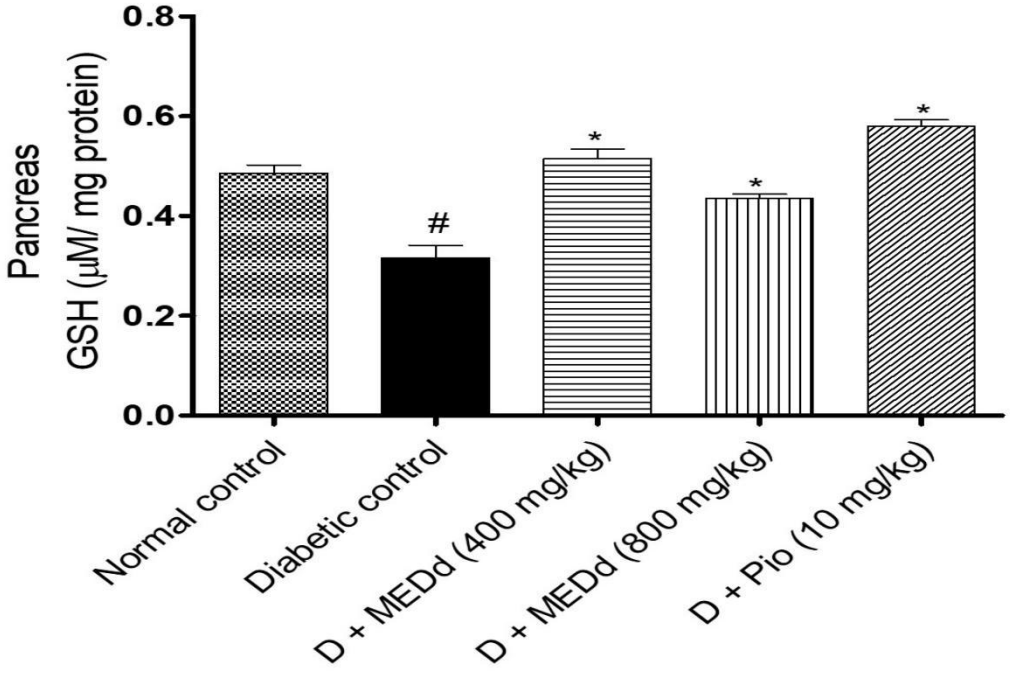

Fig. 11: Pancreatic GSH level

All values are expressed as Mean ± standard error of mean, (n=5), *p< 0.05 when compared with the diabetes control while $^{\#}p$< 0.05 was significant when compared with the normal control. D = streptozotocin induction, MEDd = methanol extract of *Dryopteris dilatata,* Pio = Pioglitazone.

Liver antioxidants

Liver MDA and Nitrite level were significantly elevated in diabetes control animals. However, treatment with MEDd (400 and 800mg/kg) and pioglitazone significantly down-regulated MDA and Nitrite levels when compared with diabetes control animals. Liver GSH level was significantly reduced following STZ induction as shown in fig. 12 and fig, 13. However, MEDd (800mg/kg) and pioglitazone significantly upregulated liver GSH level in comparison with diabetes control while MEDd (400mg/kg) did not show any significant difference in liver GSH level when compared with diabetes control animals (fig. 14).

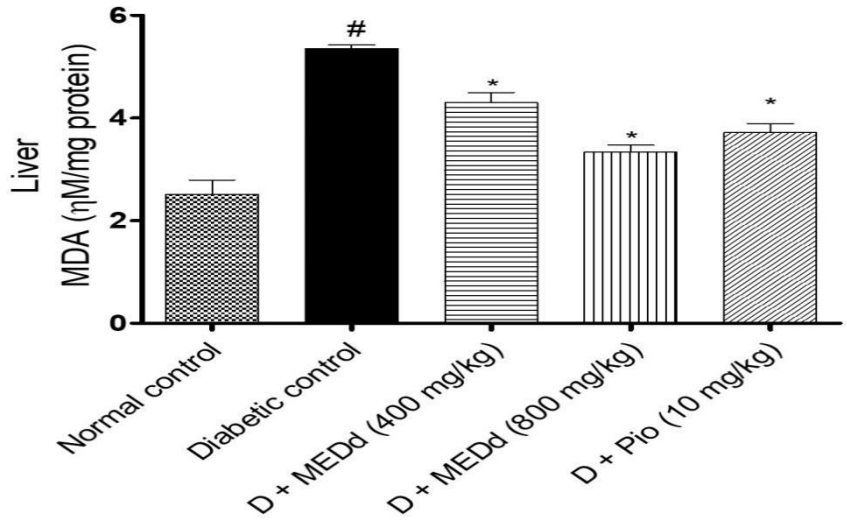

Fig. 12: liver MDA level

All values are expressed as Mean ± standard error of mean, (n=5), *p< 0.05 when compared with the diabetes control while $^{\#}p$< 0.05 was significant when compared with the normal control. D = streptozotocin induction, MEDd = methanol extract of *Dryopteris dilatata,* Pio = Pioglitazone.

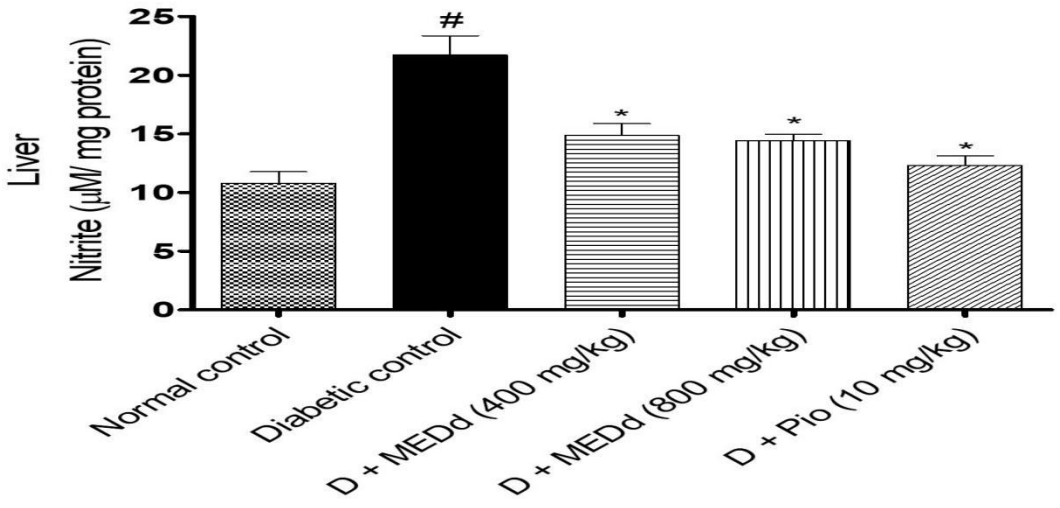

Fig. 13: liver Nitrite level

All values are expressed as Mean ± standard error of mean, (n=5), *p< 0.05 when compared with the diabetes control while $^{\#}p$< 0.05 was significant when compared with the normal control. D = streptozotocin induction, MEDd = methanol extract of *Dryopteris dilatata,* Pio = Pioglitazone.

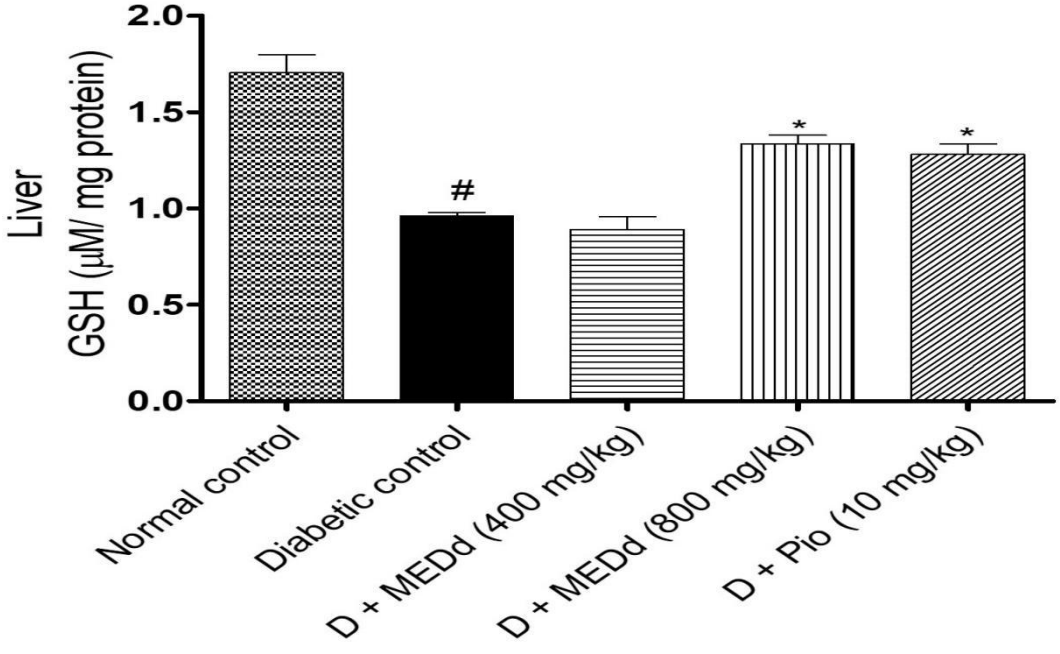

Fig. 14: liver GSH level

All values are expressed as Mean ± standard error of mean, (n=5), *p< 0.05 when compared with the diabetes control while $^{\#}p$< 0.05 was significant when compared with the normal control. D = streptozotocin induction, MEDd = methanol extract of *Dryopteris dilatata,* Pio = Pioglitazone.

Discussion

Retrospective research have showed that diabetes is a major worry that causes a variety of negative effects in the body, particularly in the brain, liver, kidneys, cardiovascular system, and musculoskeletal system. This effect could be due to uncontrollable reactive oxygen species (ROS) generation, which alters gene morphology, triggering gene mutations, and resulting in the synthesis of undesired proteins [30]. Furthermore, flavonoids and bioactive substances have been found to reduce metabolic dysregulation in animal models of diabetes and other problems in the past [13, 15]. The findings of this study on the methanol extract of *Dryopteris dilatata* (MEDd) corroborated previous reports on its ability to treat diabetes and associated consequences [31, 24, 32]. Our research found evidence that *Dryopteris dilatata* therapy can reduce the risk of diabetic complications such as hyperglycemia and hyperlipidaemia. *Dryopteris dilatata* includes bioactive phytochemicals/compounds discovered in a recent study[24] that can help to improve health by avoiding oxidative damage to cells [31, 24]. Several research on tropical plants have discovered a strong link between antioxidant activity and polyphenol levels [33, 34, 35, 25]. Repeated oral treatment with MEDd, on the other hand, was found to reduce the effects of streptozotocin (STZ)-induced oxidative stress and hyperglycemia in this investigation. In STZ-induced diabetic rats, the glucose-lowering impact of repeated oral therapy with MEDd was considerable. In the meantime, a single dose of MEDd did not significantly reduce STZ-induced hyperglycemia in diabetic rats. We also discovered that after putting diabetic rats through an oral glucose test for 30, 60, 90, 120, and 150 minutes, MEDd was able to reverse the hyperglycemic condition. In addition, the STZ-induced diabetic rats' glucose levels were favourably modulated after week 1 and 2 of treatment compared to baseline and 72 hours after streptozotocin injection. Normoglycemia is a major aim in diabetes that must be met in order to avoid organ damage and inflammation caused by persistently high blood sugar levels [36, 15, 25, 37] Streptozotocin is an antibiotic that inhibits the production of insulin, a peptide hormone responsible for the conversion of glucose to glycogen in the liver, by

targeting the beta cells of the pancreas. Reduced insulin production or insulin receptor insensitivity have been linked to impaired glucose utilization [25, 37, 22]. In line with prior research, our investigation found a drop in serum insulin in the diabetes control group, resulting in the diabetes phenotype seen in this study. Treatment with MEDd and Pioglitazone, on the other hand, increased serum insulin levels, which could be attributable to Dryopteris dilatata's antioxidant impact on pancreatic beta cells [32]. Poor blood glucose levels have also been connected to the uncontrollable development of diabetes complications such as neuropathies, cardiovascular and renal dysfunction, and cognitive and behavioral impairment, according to reports [38, 36, 15]. Several plant items have been utilized as nutraceuticals to help diabetics control their blood glucose levels [39, 31, 40, 24, 42]. As a result, our findings suggest that *Dryopteris dilatata* could be utilized as a nutraceutical in diabetes patients to help them control their blood sugar levels.

The etiology of diabetic vascular disease has been linked to hyperglycemia-induced oxidative stress [42]. However, in diabetes, the production of reactive oxygen species (ROS) due to hyperglycemia causes oxidative and inflammatory stress in a variety of organs and tissues [15]. The unbalanced production of free radicals and the diminished endogenous antioxidant defense system are the most common causes of oxidative and inflammatory stress [43, 44]. Increased reactive oxygen species (ROS) combined with a decrease in the body's natural antioxidants increase cell and tissue damage, accelerating the development of diabetic complications [45]. The use of STZ to induce diabetes in rats resulted in significant alterations in oxidative stress indicators in the rats' pancreas and liver. Oxidative stress, on the other hand, disrupts oxidative and antioxidative system functions, which is linked to cellular lipid peroxidation [27]. Organ toxicity is linked to an increase in ROS production that exceeds the cellular capacity to eliminate toxicants (Rashid *et al.,* 2013). The increased nitrite and malondialdehyde (MDA) levels in the pancreas and liver of STZ-induced diabetic mice were dramatically lowered by MEDd in this investigation. In our work, glutathione, an antioxidant activity marker, was shown to be dramatically reduced in the pancreas and liver of STZ-induced diabetic rats, implying a large increase in oxidative stress caused by ROS. The inactivation produced by excessive free radical production in hyperglycemic rats may explain the lower amount of GSH reported in diabetic control rats [15]. Additionally, due to exacerbated O_2^- and H_2O_2 levels, this GSH reduction increased nitrite and malondialdehyde levels in the pancreas and liver. Meanwhile, MEDd treatment reduced GSH loss in diabetic rats' pancreatic and liver. This demonstrated that the extract had free radical scavenging and

antioxidant capabilities. When these effects are added together, the results confirm the antioxidant potentials of *Dryopteris dilatata* ethanol extract previously reported [24]. Streptozotocin-induced changes in metabolic activities and functions in rats on biomarkers of hepatic functions were reversed in animals treated with MEDd. Streptozotocin induced significant liver damage in diabetic rats, as evidenced by increased Alanine amino transferase (ALT), Aspartate transaminase (AST), and Alkaline Phosphatase (ALP) activity. Treatment with MEDd, on the other hand, reduced the levels of liver damage indicators in the diabetic rats' plasma. One of the most common diabetic consequences is hepatotoxicity. Diabetes caused hepatic damage, which resulted in uncontrolled glucogenesis and elevated protein levels. In diabetics, the enzymes ALT, AST, and ALP have been linked to the conversion of amino acids to keto acids [46, 47]. However, the hepato-protective effect of *Dryopteris dilatata* methanol extract seen in this investigation could be due to its antiradical scavenging potential, as evidenced by reduced hepato-pancreatic lipid peroxidation (NO and MDA levels) and enhanced hepato-pancreatic GSH levels. After STZ administration, this action also reduced the increased relative liver and kidney weight produced by hyperglycemia in diabetic rats. Furthermore, increasing liver and kidney weight in patients and animal models is widely considered as an indicator of diabetes [15, 48, 25]. However, scavenging reactive oxygen species in diabetic-induced rats is likely to be the most effective defence mechanism offered by this therapeutic plant (methanol extract of Dryopteris dilatata). *Dryopteris dilatata* is capable of alleviating hyperglycemia and lowering liver toxicity in diabetic patients due to all of these effects.

Conclusion

In conclusion, the current investigation proved Dryopteris dilatata's medicinal potential in the treatment of blood glucose changes. However, increased in-vivo antioxidant activity in the pancreas and liver facilitate this anti-diabetogenic and hepatoprotective action. The phytocompounds found in the *Dryopteris dilatata* leaf may also be responsible for these activities.

Declarations

Data Availability: The data are available on request

Human and Animal Rights

The PAMO University of Medical Sciences Animal Research Ethics Committee, which agreed with the "Guide to the Care and Use of Laboratory Animals in Research and

Teaching" as prescribed in NIH publications volume 25 No.28 revised in 1996, approved the use of animals for this study with approval number PUMS-AREC/056.

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