Nutrire (2023) 48:1
https://doi.org/10.1186/s41110-022-00186-4

RESEARCH

Dryopteris dilatata leaf extract ameliorates streptozotocin-induced diabetic nephropathy in male Wistar rat

Jerome Ndudi Asiwe[1,2] · Emuesiri Goodies Moke[3] · Nicholas Asiwe[4] · Godwin D. Yovwin[5] · Bartholomew Chukwuebuka Nwogueze[2] · Tarela Melish Elias Daubry[2]

Received: 26 September 2022 / Accepted: 21 December 2022

Abstract

Background Diabetic nephropathy (DN) is a serious consequence of diabetes mellitus (DM), and it is linked to higher morbidity and mortality in diabetic patients. The quest for cheap therapeutic strategy with lesser side effects remains a major health concern. However, *Dryopteris dilatata* is a commonly found flavonoid-rich plant with plethora of therapeutic potentials. This study investigated the effect of methanol extract of *D. dilatata* (MEDd) on streptozotocin-induced diabetic nephropathy in male Wistar rat.

Methods Animals were randomly selected into five groups ($n=5$) and were treated as follows; group 1 received distilled water (10 mL/kg), group 2 received only STZ (60 mg/kg), groups 3 and 4 received STZ then 400 and 800 mg/kg of MEDd, respectively, while group 5 received STZ then pioglitazone (10 mg/kg). Following 14 days of treatment, animals were euthanized, and blood as well as pancreas and kidney tissues were collected for further studies.

Results Our results revealed that MEDd significantly reduced STZ-induced hyperglycemia in diabetic rats. Markers of oxidative injury (MDA, nitrite, and GSH) were also significantly ameliorated in the pancreas and kidney of the diabetic rats following treatment with MEDd.

However, renal function markers (creatinine and urea) were significantly attenuated with marked decreased in organ weight in the diabetic rats after treatment with MEDd. Also, serum insulin and corticosterone levels were restored following MEDd treatment.

Conclusion Methanol extract of *D. dilatata* demonstrated anti-diabetogenic and reno-protective potential by enhancing in vivo reno-pancreatic antioxidant defense system.

Keywords *Dryopteris dilatata* · Streptozotocin · Diabetes mellitus · Antioxidants · Diabetic nephropathy · Renal function

Introduction

Diabetic nephropathy (DN) is one of the most common and a serious consequence of diabetes mellitus (DM), and it is linked to higher morbidity and mortality in diabetic patients [1]. The number of diabetic individuals commencing therapy for end-stage renal disease (ESRD) in the USA increased dramatically from over 40,000 in 2000 to over 50,000 in 2020 [2]. The incidence and prevalence of DN have also risen considerably in China during the last decade. China has 24.3 million diabetic people with chronic kidney disease (CKD) [3]. Overall, the global prevalence of diabetes is rapidly increasing, particularly in emerging nations [4]. If there is no immediate change in the clinical strategy for DN prevention, the prevalence of DN is expected to rise in tandem with the rising prevalence of diabetes [5, 6]. In

✉ Jerome Ndudi Asiwe
asiwejerome@yahoo.com

1 Department of Physiology, PAMO University of Medical Sciences, Port Harcourt, Nigeria

2 Department of Physiology, Delta State University, Abraka, Nigeria

3 Department of Pharmacology, Delta State University, Abraka, Nigeria

4 Department of Anatomy, University of Port Harcourt, Port Harcourt, Nigeria

5 Department of Family Medicine, Delta State University, Abraka, Nigeria

Published online: 28 December 2022

about one-third of diabetic patients, DN develops after a latency phase that can last many years. Individuals should be evaluated for microalbuminuria or screened to anticipate DN, known as the customized medicine approach, so that resources with more extensive therapy and early preventive interventions can be allocated solely to those who are most at risk [7]. There has been extensive glomerulopathy at the time of microalbuminuria [8, 9]; however, a considerable proportion of patients with microalbuminuria can regress to normal albuminuria [8–10]. The problem of diagnosing diabetic nephropathy independent of retinopathy which has a prevalence of 40%, as well as a number of patients with DN who do not match the conventional pattern of DN [11–13]. Type 2 DM patients are more likely to have non-proteinuric DN and DN without retinopathy. Because RAS blockade therapy is usually started only after chronic albuminuria, the absence of albuminuria can make it difficult to know whether to start extensive therapeutic efforts [7].

The pathophysiology of DN is extremely complex, and the cause is still unknown, resulting in poor therapy success. Standard therapy, which includes rigorous blood sugar and blood pressure control, has been demonstrated to be ineffective in preventing DN progression to ESRD and DN-related mortality [14, 15]. In order to create innovative techniques for treating DN, it is critical to improve our understanding of the pathophysiology of DN and research into the pathogenic mechanisms of the disease. Many routes and mediators are implicated in the development and progression of DN [16], including oxidative stress, angiotensin II (Ang-II), and inflammatory processes, all of which are now thought to play a key role [17]. The fact that inhibiting oxidative stress improves a characteristic associated with streptozotocin-induced DN has highlighted the involvement of oxidative stress in DN [18]. Traditionally, oxidative stress is defined as tissue oxidative damage caused by an imbalance of oxidants and antioxidants [19]. Many of the processes involved in the pathophysiology of DN including hyperglycemia itself produce oxidative stress [15]. The pathophysiology of DN is linked to an increase in reactive oxygen species (ROS) caused by hyperglycemia. Understanding the main characteristics of the oxidative stress-induced path mechanism involved in the formation and progression of DN enables for the discovery of new potential targets and the development of novel antioxidant treatment methods [17].

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 [20]. The Urhobo tribe in Nigeria affectionately refers to it as Okpomie, and it is primarily found in Nigeria's tropical region [21, 22]. 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 [20]. We also discovered that an ethanol extract of the plant's leaves had anti-hyperlipidemic and anti-diabetic properties [22]. Our previous research also demonstrated that plant leaves contain a large number of phytochemicals and active substances [22]. The impact of a methanol extract of *D. dilatata* (MEDd) on type 1 diabetes mellitus-induced renal impairment in male Wistar rats was examined in this study.

Materials and procedures

Chemicals and drugs

Santa Cruz (USA) provided the streptozotocin, while pioglitazone produced by LEK S.A. (U1, Podlipie 16, PL-95–010 Strykow, Poland) was acquired at a neighborhood pharmacy in Port Harcourt, Nigeria. Sigma Aldrich (Germany) provided thiobarbituric acid (TBA), Griess, and Ellman reagents. Burgoyne Burbidges Co. (Mumbai, India) provided the trichloroacetic acid (TCA), and Immunometrics Limited provided the insulin ELISA kit, creatinine and urea assays (Randox kits) (UK). The rest of the reagents and solvents were of analytical quality.

Animals care

Twenty-five mature male Wistar rats weighing 120–150 g were purchased from the central animal house, PAMO University of Medical Sciences, Port Harcourt, Nigeria. The animals were kept in regular laboratory conditions as per the University's ethical guidelines (PUMS-AREC/2021/067), which adhere to the "Principle of Laboratory Animal Care" (NIH Publication No. 85–23). The rats were given free access to the regular rat chow (Ladokun feeds) and water.

Collection, identification, and extraction of plants

Dryopteris dilatata fresh leaves were obtained from the Olomoro community in Isoko South, Delta State, Nigeria. The leaves were authenticated for herbarium numbering, FHI 110,338, at the Forestry Research Institute of Nigeria (FRIN), Ibadan. Before extraction, the leaves were washed, air-dried, and macerated into powder form. Furthermore, the Alawode and colleagues [23] plant crude extraction methods were adopted and modified. A total of 70% methanol was used to extract the mixed powder which was then filtered using Whatman No. 2 filter paper, and then, rotary evaporator was used to vacuum concentrate the solvent to dryness. Finally, to obtain the required dose of 800 mg/kg, the dried extract was further dissolved with distilled water. The phytochemical screening of *D. dilatata* was previously done and reported by Akpotu et al. [22].

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. [24]. The rats' diabetic status was determined after 72 h using a glucometer (ACCU-CHEK® Active) and appropriate blood glucose test strips. Animals with a fasting blood glucose level of more than or equal to 200 mg/dl (≥ 200 mg/dl) were selected for the study [24].

Design of the treatment

The animals were divided into five groups ($n = 5$) at random. Non-diabetic treated rats (normal control rats) received distilled water (10 mL/kg) in group 1. Groups 2–5 were given STZ 60 mg/kg body weight and were diagnosed as diabetics 72 h after STZ induction. However, group 2 served as the negative control rats (diabetic control rats), receiving distilled water (10 mL/kg); groups 3 and 4 received 400 mg/kg and 800 mg/kg of methanol extract of *D. dilatata* (MEDd), respectively. This dose was based on the findings of our previous report and preliminary investigation [25], and group 5 served as positive control and received 10 mg/kg of pioglitazone (standard anti-diabetic drug). Oral gavage was used in all treatments throughout the period of the experiment (2 weeks). The animals were observed for mortality, and fasting blood sugar from the tail vein was evaluated from overnight fasted rats at 7 days interval for the duration of the study with a glucometer (Accu-check® Active, Roche diagnostic, Mannheim Germany). The change in body weight was monitored excluding day 0.

Biochemical study of blood samples and tissue homogenates

The rats were given deep ether anesthesia via inhalation before being euthanized via cervical dislocation at the conclusion of the treatment period. Blood was collected through cardiac puncture, and plasma was obtained using a benchtop centrifuge (Bosch, UK) at 3000 rpm at room temperature. The pancreas, spleen, liver, and kidney were removed and washed in ice-cold phosphate buffer saline (0.1 M, pH 7.4) before being blotted with adsorbent paper. In addition, the kidney and pancreas were homogenized in cold phosphate buffer saline (0.1 M, pH 7.4) and centrifuged for 10 min at 4 °C at 10,000 rpm. The tissue supernatant (pancreas and liver) was collected for biochemical examination and utilized to calculate malondialdehyde (MDA), nitrite, and reduced glutathione (GSH). The serum was also used to estimate renal function tests.

Estimation of levels of lipid peroxidation

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

Measurement of tissue nitrite

Spectrophotometric methods using Griess reagent were used to quantify nitrite in the pancreas and kidney. Griess reagent was made in a 1:1 ratio from reagents A (1% sulfanilamide in 5% phosphoric acid) and B (0.1% 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 reduced glutathione

The amount of reduced GSH in pancreas and kidney supernatants was determined using Ellman's reagent in a modified approach [27]. 0.1 mL cell-free exudate supernatant was diluted 10 times and deproteinized with 1 mL trichloroacetic acid (20%) before centrifugation at 10,000 rpm for 10 min 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 min (752 N INESA, China). The glutathione concentration was calculated using a standard curve constructed using standard glutathione (0–200 M) and expressed as a percentage of total glutathione per milligram protein.

Kidney function markers estimation

The concentrations of serum creatinine and urea were determined using a Randox test kit, as described by Reitman and Frankel [28].

Serum insulin estimation

Insulin and corticosterone concentrations in the blood were measured using their ELISA kits (Elabscience, UK) according to the manufacturer's instructions.

Statistical analysis

All data was analyzed using one-way and two-way analysis of variance (ANOVA) with Bonferroni post hoc multiple comparison testing. It was determined that $P < 0.05$ was statistically significant. GraphPad Prism software version 5.01 (GraphPad Software, Inc. La Jolla, CA 92,037, USA) was used to create graphs and conduct statistical analysis.

Results

Change in body weight

There was no significant change in the body weight of animals at week 0. However, at weeks 1 and 2, diabetes control animals showed a reduced body weight, but following treatment with MEDd and pioglitazone at weeks 1 and 2, the body weight was significantly normalized [treatment: $F(8, 75) = 9.71$; $p < 0.0001$, interaction: $F(4, 75) = 25.48$; $p < 0.0001$ and interaction × treatment: $F(2, 75) = 3.26$; $p = 0.0441$] as presented in Fig. 1.

Relative weight of spleen, liver, and kidney

Figure 2 shows that there was no significant difference in the weight of the spleen across the experimental groups. However, the liver showed a marked increase in the diabetes control group. Following treatment with MEDd (400 and 800 mg/kg) and pioglitazone (10 mg/kg), the liver weight was significantly reduced [treatment: $F(8, 29) = 2.02$; $p = 0.0791$, interaction: $F(4, 29) = 14.33$; $p < 0.0001$ and interaction × treatment: $F(2, 29) = 1441.75$; $p < 0.0001$]. Similar data was observed in the weight of the kidney though MEDd (400 mg/kg) did not have any significant effect on the weight of the kidney when compared with the control.

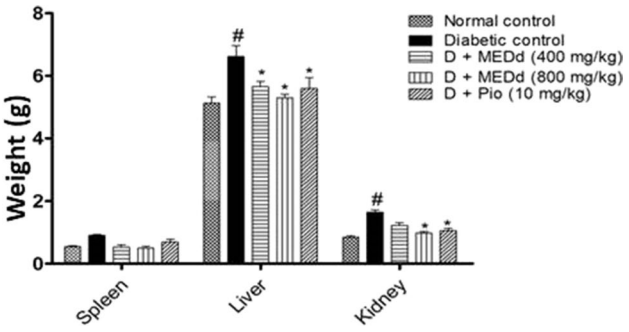

Fig. 2 Organ weight of spleen, liver, and kidney. All values are expressed as mean ± standard error of mean, ($n = 5$), $^{*}p < 0.05$ when compared with the diabetes control while $^{\#}p < 0.05$ was significant when compared with the normal control. D, streptozotocin induction; MEDd, methanol extract of *Dryopteris dilatata*; Pio, pioglitazone

Fasting blood sugar

Following the induction of diabetes with STZ, the fasting glucose level was observed to be above 200 mg/dl after 72 h of induction [$F(4, 10) = 83.92$, $p < 0.0001$]. However, treatment with MEDd (400 and 800 mg/kg) and pioglitazone significantly reduced the glucose level in a duration-dependent manner as presented in Fig. 3.

Glucose tolerance test

Following oral administration of glucose, the glucose level in the diabetes control group was significantly increased in 30, 60, 90, 120, and 150 min when compared with the normal control [treatment: $F(11, 100) = 80.32$; $p < 0.0001$, interaction: $F(4, 100) = 161.41$; $p < 0.0001$ and interaction × treatment: $F(3, 100) = 1236.39$; $p < 0.0001$]. However, treatment with MEDd (400 and 800 mg/dl) and pioglitazone (10 mg/kg) significantly reduces the glucose level in 30, 60, 90, 120, and 150 min when compared with diabetes control but was

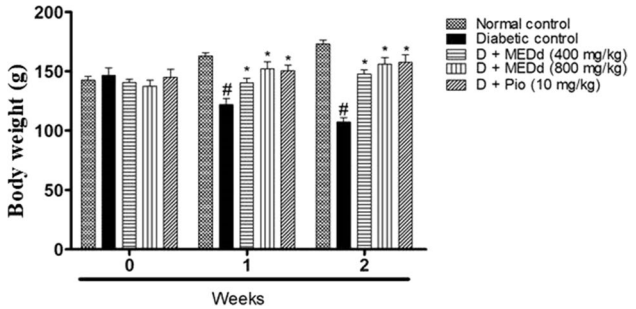

Fig. 1 Body weight (g). All values are expressed as mean ± standard error of mean, ($n = 5$), $^{*}p < 0.05$ when compared with the diabetes control while $^{\#}p < 0.05$ was significant when compared with the normal control. D, streptozotocin induction; MEDd, methanol extract of *Dryopteris dilatata*; Pio, pioglitazone

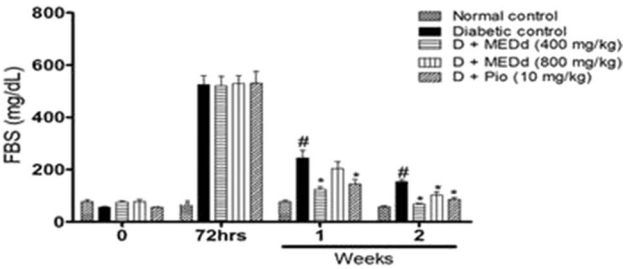

Fig. 3 Fasting blood sugar (FBS). All values are expressed as mean ± standard error of mean, ($n = 5$), $^{*}p < 0.05$ when compared with the diabetes control while $^{\#}p < 0.05$ was significant when compared with the normal control. D, streptozotocin induction; MEDd, methanol extract of *Dryopteris dilatata*; Pio, pioglitazone

significantly elevated when compared with normal control animals as shown in Fig. 4.

Serum insulin

Figure 5 represents the serum insulin concentration. The serum insulin concentration [F(4, 10) = 30.00, ($p < 0.0001$] was significantly reduced in diabetes control animal following the STZ induction. However, treatment with MEDd (400 and 800 mg/kg) and pioglitazone significantly increases the serum concentration of insulin.

Serum corticosterone

There was a significant increase in serum corticosterone [F(4, 15) = 10.39, $p = 0.0003$] in the diabetic control group when compared with the normal control group. However, treatment with MEDd (400 and 800 mg/kg) and pioglitazone significantly restored the streptozotocin-induced increase in corticosterone level as presented in Fig. 6.

Kidney function markers

Figures 7 and 8 represented the serum concentration of creatinine and urea in streptozotocin-induced nephropathy. Creatinine [F(4, 10) = 25.69, $p < 0.0001$] was significantly increased in the diabetic control group when compared with the normal control group. However, following treatment with MEDd (400 and 800 mg/kg) and pioglitazone, the values were significantly reduced (Fig. 7). Following treatment with MEDd (400 and 800 mg/kg), the serum urea level [F(4, 10) = 9.575, $p = 0.0019$] was significantly reduced against the increase caused by streptozotocin. Though STZ-induced increase was not significant when compared with the control, the pioglitazone treatment did not significantly alter the serum urea level in Fig. 8.

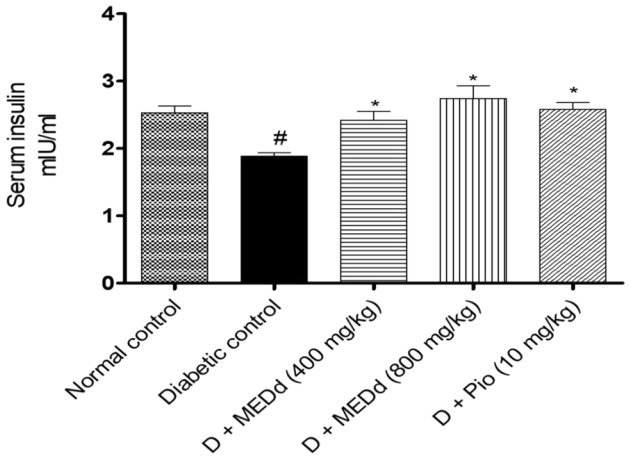

Fig. 5 Serum insulin concentration. All values are expressed as mean ± standard error of mean, ($n = 5$), $^{*}p < 0.05$ when compared with the diabetes control while $^{\#}p < 0.05$ was significant when compared with the normal control. D, streptozotocin induction; MEDd, methanol extract of *Dryopteris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

Pancreatic oxidative stress

Presented in Figs. 9, 10, and 11 is the pancreatic oxidative stress marker. There was a 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 800 mg/dl) and pioglitazone (10 mg/kg) significantly reduces the MDA [F (4, 10) = 21.01, $p < 0.0001$] as well as elevated the pancreatic GSH level

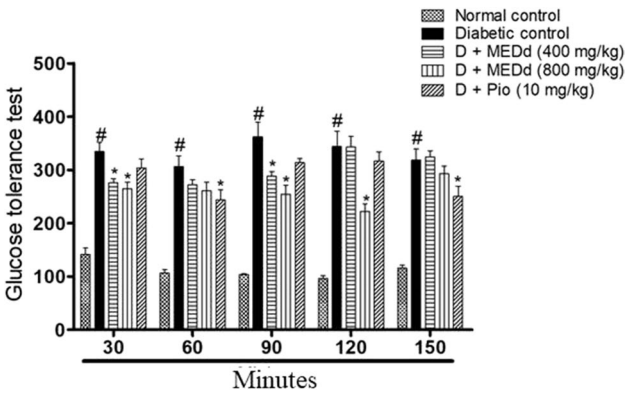

Fig. 4 Oral glucose tolerance (OGT). All values are expressed as mean ± standard error of mean, ($n = 5$), $^{*}p < 0.05$ when compared with the diabetes control while $^{\#}p < 0.05$ was significant when compared with the normal control. D, streptozotocin induction, MEDd, methanol extract of *Dryopteris dilatata*; Pio, pioglitazone

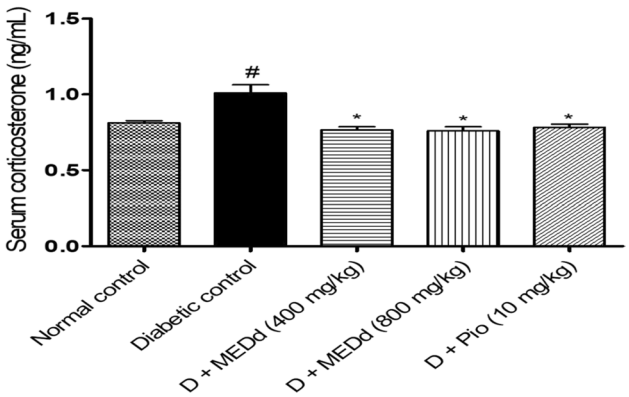

Fig. 6 Serum corticosterone. All values are expressed as mean ± standard error of mean, ($n = 5$), $^{*}p < 0.05$ when compared with the diabetes control while $^{\#}p < 0.05$ was significant when compared with the normal control. D, streptozotocin induction; MEDd, methanol extract of *Dryopteris dilatata*; Pio, pioglitazone

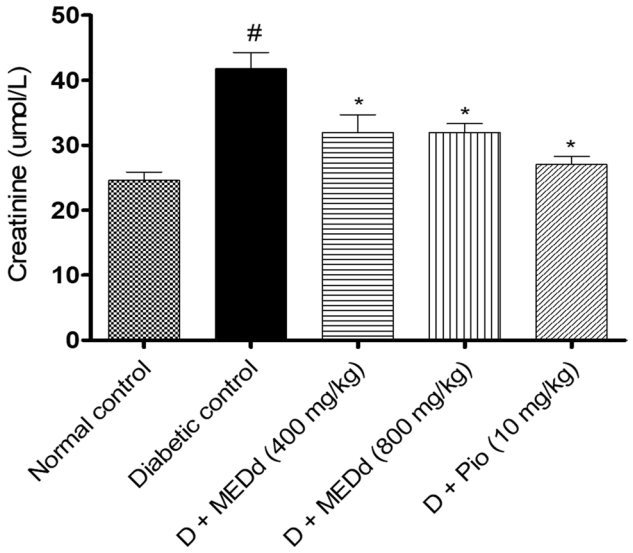

Fig. 7 Serum creatinine level. All values are expressed as mean ± standard error of mean, ($n = 5$), $^{*}p < 0.05$ when compared with the diabetes control while $^{\#}p < 0.05$ was significant when compared with the normal control. D, streptozotocin induction; MEDd, methanol extract of *Dryopteris dilatata*; Pio, pioglitazone

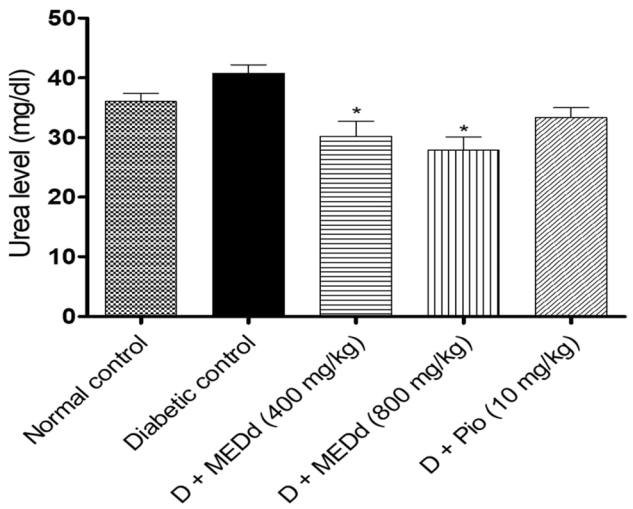

Fig. 8 Serum urea level. All values are expressed as mean ± standard error of mean, ($n = 5$), $^{*}p < 0.05$ when compared with the diabetes control while $^{\#}p < 0.05$ was significant when compared with the normal control. D, streptozotocin induction; MEDd, methanol extract of *Dryopteris dilatata*; Pio, pioglitazone

[F(4, 10) = 8.007, $p = 0.0037$] when compared to diabetes control animal. Treatment with MEDd (400 and 800 mg/kg) did not show any significant difference in pancreatic nitrite level [F (4, 10) = 10.40, $p = 0.0614$] when compared with diabetes control animal.

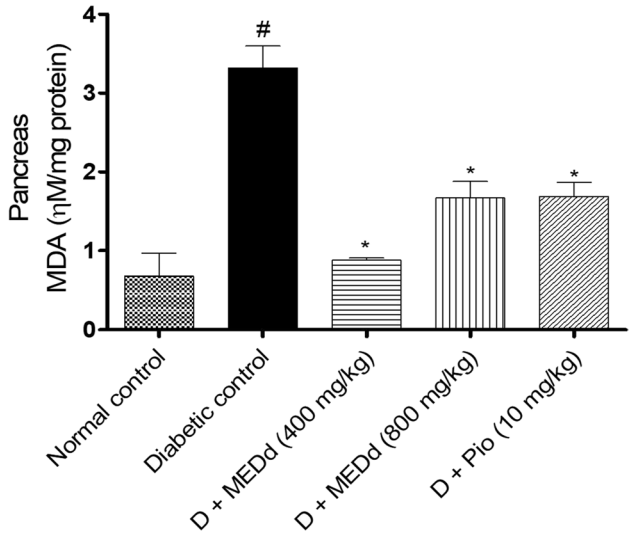

Fig. 9 Pancreatic MDA level. All values are expressed as mean ± standard error of mean, ($n = 5$), $^{*}p < 0.05$ when compared with the diabetes control while $^{\#}p < 0.05$ was significant when compared with the normal control. D, streptozotocin induction; MEDd, methanol extract of *Dryopteris dilatata*; Pio, pioglitazone

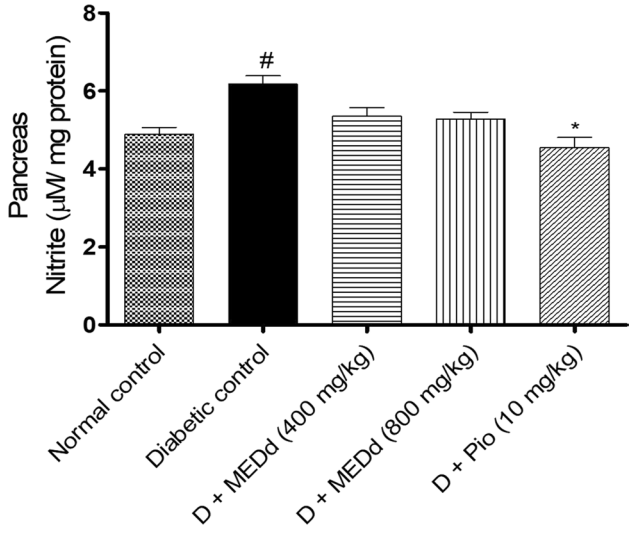

Fig. 10 Pancreatic nitrite level. All values are expressed as mean ± standard error of mean, ($n = 5$), $^{*}p < 0.05$ when compared with the diabetes control while $^{\#}p < 0.05$ was significant when compared with the normal control. D, streptozotocin induction; MEDd, methanol extract of *Dryopteris dilatata*; Pio, pioglitazone

Kidney oxidative stress

Kidney oxidative stress markers were assayed and presented in Figs. 12, 13, and 14. They were significant increase in kidney MDA [F(4, 10) = 14.31, $p = 0.0004$] and nitrite [F(4, 10) = 6.041, $p = 0.0097$] following

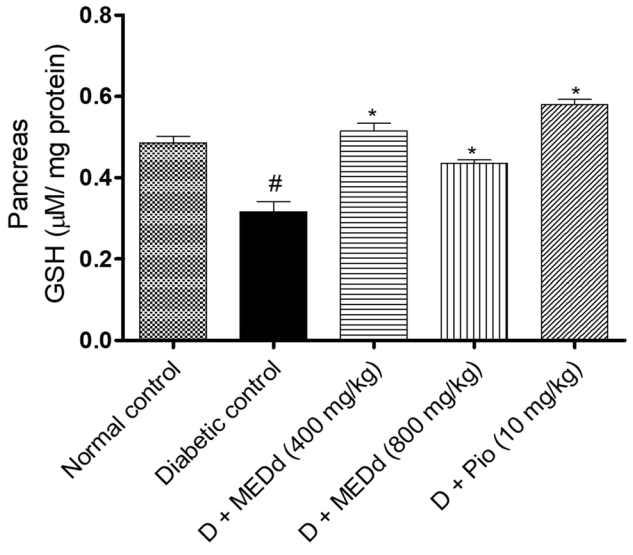

Fig. 11 Pancreatic GSH level. All values are expressed as mean ± standard error of mean, ($n = 5$), $^{*}p < 0.05$ when compared with the diabetes control while $^{\#}p < 0.05$ was significant when compared with the normal control. D, streptozotocin induction; MEDd, methanol extract of *Dryopteris dilatata*; Pio, pioglitazone

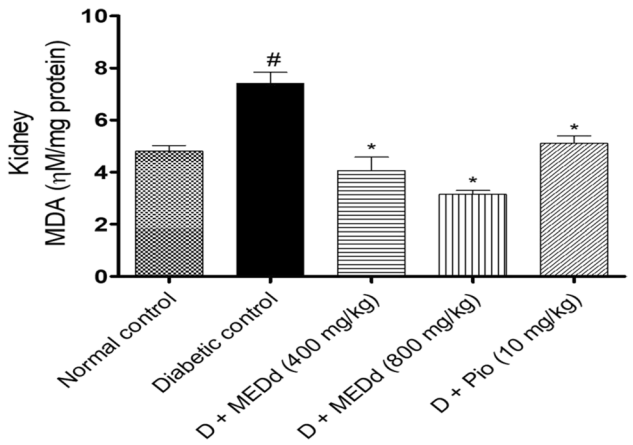

Fig. 12 Kidney malondialdehyde (MDA) level. All values are expressed as mean ± standard error of mean, ($n = 5$), $^{*}p < 0.05$ when compared with the diabetes control while $^{\#}p < 0.05$ was significant when compared with the normal control. D, streptozotocin induction; MEDd, methanol extract of *Dryopteris dilatata*; Pio, pioglitazone

STZ induction. However, treatment with MEDd (400 and 800 mg/kg) and pioglitazone significantly lowered the MDA (Fig. 12) but had no significant effect on the kidney nitrite (Fig. 13). Kidney GSH [$F(4, 10) = 24.20$, $p < 0.0001$] was significantly reduced in the diabetic control group when compared with the normal control group. However, treatment with MEDd (400 and 800 mg/kg) and pioglitazone significantly increased the kidney GSH level (Fig. 14).

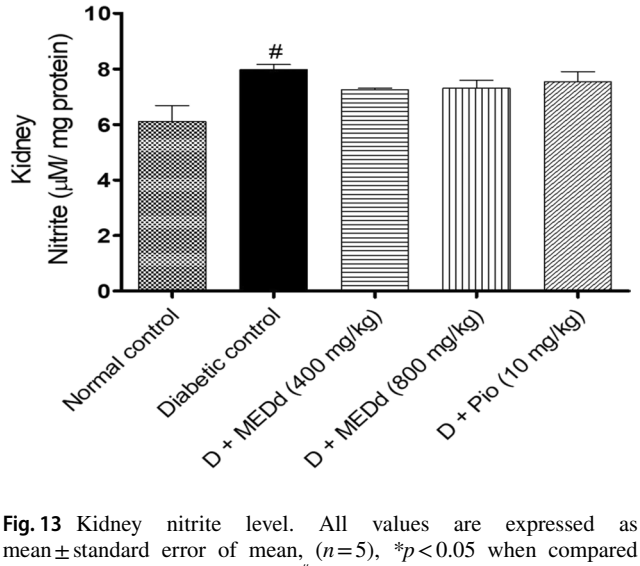

Fig. 13 Kidney nitrite level. All values are expressed as mean ± standard error of mean, ($n = 5$), $^{*}p < 0.05$ when compared with the diabetes control while $^{\#}p < 0.05$ was significant when compared with the normal control. D, streptozotocin induction; MEDd, methanol extract of *Dryopteris dilatata*; Pio, pioglitazone

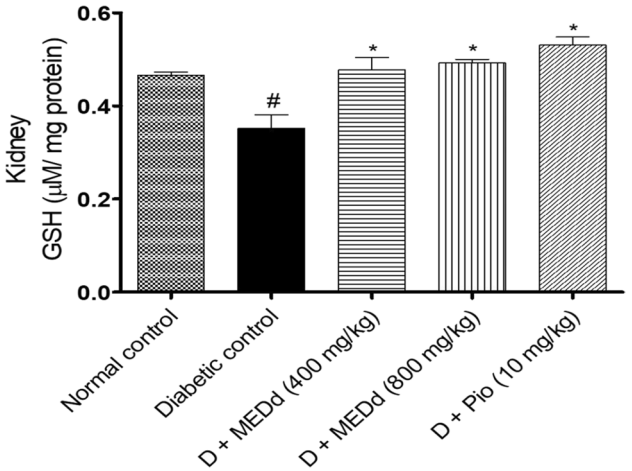

Fig. 14 Kidney glutathione (GSH) level. All values are expressed as mean ± standard error of mean, ($n = 5$), $^{*}p < 0.05$ when compared with the diabetes control while $^{\#}p < 0.05$ was significant when compared with the normal control. D, streptozotocin induction; MEDd, methanol extract of *Dryopteris dilatata*; Pio, pioglitazone

Discussion

The purpose of this study was to see how methanol extract of *D. dilatata* (MEDd) affected streptozotocin-induced diabetic nephropathy in male Wistar rats. The results showed that methanol extract of *D. dilatata* reduced the effects of streptozotocin-induced diabetes on renal function. Diabetic mellitus is a big worry, according to retrospective research, as it 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 ROS generation, which alters gene morphology, triggering gene mutations and resulting in the synthesis of undesired proteins [29]. Nevertheless, flavonoids and bioactive substances have been found to reduce metabolic dysregulation in animal models of diabetes and other problems in the past [23, 30–32]. The findings of this study on the methanol extract of *D. dilatata* (MEDd) corroborated previous reports on its ability to treat diabetes and its associated consequences [21, 22].

Our research found evidence that *D. dilatata* medication can reduce the risk of diabetic complications such as reno-inflammation and hyperglycemia. *D. dilatata* includes bioactive phytochemical substances that can improve health by avoiding cellular inflammation as reported in a prior study [21, 22]. Flavonoids, a powerful phenolic molecule that functions as a free radical scavenger, can scavenge stable free radicals that are normally associated with high antioxidant activity [33, 34]. Several studies of tropical plants have discovered a strong link between antioxidant activity and polyphenol levels [32, 35, 36]. Repeated oral therapy with MEDd, on the other hand, reduced the effects of streptozotocin-induced oxidative stress and hyperglycemia, according to this study. In streptozotocin-induced diabetic rats, the glucose-lowering impact of MEDd was significant after repeated oral therapy. Meanwhile, in diabetic rats, a single dose of MEDd had no effect on STZ-induced hyperglycemia. MEDd was also found to be able to correct hyperglycemia in diabetic rats following an oral glucose test lasting 30, 60, 90, 120, and 150 min. Furthermore, when compared to baseline and 72 h after streptozotocin injection, glucose levels in STZ-induced diabetic rats were favorably modified after weeks 1 and 2. In diabetes, normoglycemia is a critical objective to achieve in order to avoid organ damage and inflammation caused by high blood sugar levels [31, 37, 38]. 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 [31, 37–39]. Several plant items have been utilized as nutraceuticals to help diabetics control their blood glucose levels [21, 22, 40, 41]. As a result, our findings suggest that *D. 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 [42–44]. However, in diabetes, the rapid production of ROS due to hyperglycemia causes oxidative and inflammatory stress in a variety of organs and tissues. The etiology of diabetic vascular disease is thought to be linked to hyperglycemia-induced oxidative stress [42–44]. In diabetes, however, hyperglycemia causes oxidative and inflammatory stress in different organs and tissues. The unbalanced generation of free radicals and a reduced endogenous antioxidant defense system are the most common causes of oxidative and inflammatory stress [24, 45, 46]. Increased ROS combined with a decrease in the body's natural antioxidants increase cell and tissue damage, accelerating the development of diabetic complications [44, 47]. In diabetic control rats, corticosterone, a glucocorticoid released in the zona fasciculata of the adrenal cortex, was found to considerably increase, indicating inflammatory stress. However, streptozotocin-induced diabetes in rats resulted in considerable changes in oxidative stress indicators in the pancreas and kidneys. Oxidative stress, on the other hand, disrupts the oxidants and antioxidants delicate balance which is associated with cellular lipid peroxidation [43]. Organ toxicity is linked to an increase in ROS production that exceeds the cellular capacity to eliminate toxicants [47]. The high serum corticosterone level as well as malondialdehyde and nitrite levels in the pancreas and kidney of streptozotocin-induced diabetic rats was considerably lowered by MEDd in this investigation. Notably, this effect could be attributed to the anti-inflammatory and anti-lipid peroxidative damage of the plant as previously reported by Akpotu et al. [22]. Glutathione is an important defensive marker that indicates oxidative stress when the values are reduced [31, 48]. The inactivation produced by excessive free radical production in hyperglycemic rats could explain the lower levels of GSH level observed in diabetic control rats [31]. Furthermore, decreased GSH level resulted in an increase in malondialdehyde levels in the pancreas and kidney, which is attributable to increased O_2^- and H_2O_2. MEDd administration, on the other hand, boosted the antioxidant powers. 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 *D. dilatata* methanol extract previously reported [22]. Streptozotocin-induced changes in metabolic activities and functions on indicators of renal function in rats were reversed in animals treated with MEDd. Oxidative stress causes increased protein breakdown, which boosts ammonia levels and as a result, serum urea concentrations [34, 48]. As a result of the disintegration of the brush border epithelia of renal cells caused by free radicals, the cells became impermeable to urea and creatinine [34]. Due to restricted or no tubular absorption of urea and creatinine by the renal tubules, the levels of these kidney markers in the blood rise. Treatment with MEDd, on the other hand, reduced kidney damage indicators in the diabetic rats' plasma. One of the most prevalent diabetes consequences is nephropathy, and measuring serum urea and creatinine concentrations for indicators of renal impairment is well known [49]. The free radical

scavenging potential of the methanol extract of *D. dilatata*, as evidenced by reduced reno-pancreatic lipid peroxidation (MDA and nitrite levels) and higher reno-pancreatic GSH, could explain the reno-protective effect observed in this investigation. After streptozotocin therapy, 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 [31, 50]. Nonetheless, the most effective defense mechanisms demonstrated by this medicinal agent: methanol extract of *D. dilatata* would probably be proposed as scavenging reactive oxygen species in diabetic-induced rats. With all of these effects, *D. dilatata* can help to reduce hyperglycemia and renal dysfunction in diabetics.

Conclusion

Finally, this study found that administration of methanol extracts of *D. dilatata* ameliorated renal dysfunctions caused by diabetes mellitus by considerably lowering serum urea and creatinine levels in response to a streptozotocin-induced increase. By significantly upregulating reno-pancreatic antioxidant defense systems, *D. dilatata* appeared to be a natural key to reno-pancreatic protection against oxidative damage as a result of our biochemical analysis. However, there was no significant difference between *D. dilatata* treatment and pioglitazone. Hence this study has provided a cheap and easily available alternative therapeutic approach to diabetic complications. Also, our team is currently working on elucidating the possible molecular mechanism of these findings.

Acknowledgements The technical knowledge of Divic Specialist Diagnostic Laboratory, Port Harcourt, Rivers State, Nigeria, is gratefully acknowledged by the authors.

Author contribution The study was conceptualized by Jerome Ndudi Asiwe; the experimental assay was managed by Emuesiri Goodies Moke, Bartholomew Chukwuebuka Nwogueze, Daubry Tarela Mellish Elias, and Godwin D Yovwin; and the statistical analysis as well as manuscript writing was done by Jerome Ndudi Asiwe and Nicholas Asiwe.

Funding The study was entirely self-funded by the authors.

Data availability This manuscript includes all of the study's data.

Declarations

Competing interests The authors declare no competing interests.

Conflict of interest The authors declare no competing interests.

Human and animal rights The PAMO University of Medical Science research ethics committee (PUMS-AREC/2021/067) approved the study, and the procedure closely complied with the ARRIVE recommendations as outlined in the 1996 revision of NIH Publication No. 85–23.

References

1. Valencia WM, Florez H. "How to prevent the microvascular complications of type 2 diabetes beyond glucose control. BMJ. 2017;356:i6505.
2. Burrows NR, Hora I, Geiss LS, Gregg EW, Albright AA. "Incidence of end-stage renal disease attributed to diabetes among persons with diagnosed diabetes—United States and Puerto Rico, 2000–2014", MMWR. Morb Mortal Wkly Rep. 2017;66:1165–70.
3. Zhang L, Long J, Jiang W, et al. Trends in chronic kidney disease in China. N Engl J Med. 2016;375:905–6.
4. Xue R, Gui D, Zheng L, Zhai R, Wang F, Wang N. Mechanistic insight and management of diabetic nephropathy: recent progress and future perspective. J Diabetes Res. 2017.
5. Stenvinkel P. Chronic kidney disease: a public health priority and harbinger of premature cardiovascular disease". J Intern Med. 2010;268:456–67.
6. Gheith O, Farouk N, Nampoory N, Halim MA, Al- Otaibi T. Diabetic kidney disease: worldwide difference of prevalence and risk factors. J Nephropharmacol. 2015;5:49–56.
7. Susztak K, Böttinger EP. Diabetic nephropathy: a frontier for personalized medicine". J Am Soc Nephrol. 2006;17:361–7.
8. Fioretto P, Steffes MW, Mauer M. Glomerular structure in non-proteinuric IDDM patients with various levels of albuminuria". Diabetes. 1994;43:1358–64.
9. Caramori ML, Kim Y, Huang C, et al. Cellular basis of diabetic nephropathy: 1 study design and renal structuralfunctional relationships in patients with long-standing Type 1 diabetes. Diabetes. 2002;51(2):506–13.
10. Perkins BA, Ficociello LH, Silva KH, Finkelstein DM, Warram JH, Krolewski AS. Regression of microalbuminuria in type 1 diabetes". N Engl J Med. 2003;348:2285–93.
11. Trevisan R, Vedovato M, Mazzon C, et al. Concomitance of diabetic retinopathy and proteinuria accelerates the rate of decline of kidney function in type 2 diabetic patients". Diabetes Care. 2002;25:2026–31.
12. Kramer HJ, Nguyen QD, Curhan G, Hsu C. Renal insufficiency in the absence of albuminuria and retinopathy 10 BioMed Research International among adults with type 2 diabetes mellitus". JAMA. 2003;289:3273–7.
13. Chen Y, Lee K, Ni Z, He JC. Diabetic kidney disease: challenges, advances, and opportunities". Kidney Dis. 2020;6(4):215–25.
14. Arora MK, Singh UK. Molecular mechanisms in the pathogenesis of diabetic nephropathy: an update". Vascul Pharmacol. 2013;58:259–71.
15. Kopel J, Pena-Hernandez C, Nugent K. Evolving spectrum of diabetic nephropathy". World J Diabetes. 2019;10:269–79.
16. Tavafi M. Diabetic nephropathy and antioxidants". J Nephropathol. 2013;2:20–7.
17. Donate-Correa J, Luis-Rodríguez D, Martín-Núñez E, et al. Inflammatory targets in diabetic nephropathy". J Clin Med. 2020;9(2):458.
18. Thallas-Bonke V, Thorpe SR, Coughlan MT, et al. Inhibition of NADPH oxidase prevents advanced glycation end product-mediated damage in diabetic nephropathy through a protein kinase C- -dependent pathway". Diabetes. 2008;57(2):460–9.

19. Kao MP, Ang DS, Pall A, Struthers AD. Oxidative stress in renal dysfunction: mechanisms, clinical sequelae and therapeutic options. J Hum Hypertens. 2010;24(1):1–8.
20. Rünk K, Zobel M, Zobel K. Biological Flora of the British Isles: Dryopteris carthusiana, D. dilatata and D. expansa. J Ecol. 2012;100(4):1039–63.
21. Mordi JC, Lawrence EO, Chiedozie O. queous Leaf Extract of Dryopteris dilatata on STZ--Induced Diabetic Wistar Rats with Associated Hyperlipidemic Ameliorating Property. Journal of Dental and Medical Sciences. 2016;15(6):97–104.
22. Akpotu A, Celestine A, Choice N, Okorie P, Igwe U, Jide U, Adeyemo M, Nwaeme O, Obinna O. Antidiabetic and anti-hyperlipidemic effects of ethanolic extract of Dryopteris dilatata leaves. Journal of Diabetes and Endocrinology., 2018;9(3):20–7.
23. Alawode DI, Asiwe JN, Moke EG, Okonofua DE, Sanusi KO, Adagbada EO, Yusuf MO, Fasanmade AA. The effect of ethanol leaf extract of *Cnidosculus Aconitifolius* on cardiorenal functions in hypertensive and normotensive male Wistar rats. Int J Nutr Sci. 2021;6(3):155–60. https://doi.org/10.30476/IJNS.2021.92067.1145.
24. Asiwe JN, Anachuna KK, Moke EG, Sanusi KO, Okonofua DE, Omeru O, Fasanmade AA. High dietary salt intake alleviates fasting blood glucose in streptozotocin-induced diabetic male Wistar rats. Thai J Pharm Sci. 2021;45(3):172–7.
25. Akpotu EA, Ghasi SI, Ewhre LO, Adebayo OG, Asiwe JN. Antidiabetogenic and *in vivo* antioxidant activity of ethanol extract of *Dryopterisdilatata* in alloxan-induced male Wistar rats. Biomarkers. 2021;26(8):718–25.
26. McCord JM, Fridovich I. Superoxide dismutase. An enzymic function for erythrocuprein (hemocuprein). J Biol Chem. 1969;244:6049–55.
27. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. 1979;95:351–8.
28. Reitman S, Frankel S. Glutamic-pyruvate transaminase assay by colorimetric method. Am J Clin Path. 1957;28:56.
29. Klil-Drori AJ, Azoulay L, Pollak MN. Cancer, obesity, diabetes, and antidiabetic drugs: is the fog clearing? Nat Rev Clin Oncol. 2017;14(2):85–99.
30. Anachuna KK, Oyem CJ, Nwogueze BC, Asiwe JN. Glucose lowering effects and histomorphological changes of *Vernonia amygdalina* on pancreatic compromised Wistar rats using alloxan monohydrate. Trop J Health Sci. 2018;25(2):27–31.
31. Ajayi AM, Adedapo ADA, Badaki VB, Oyagbemi AA, Adedapo AA. Chrysophyllum albidum fruit ethanol extract ameliorates hyperglycaemia and elevated blood pressure in streptozotocin-induced diabetic rats through modulation of oxidative stress. NF-κB and PPAR-γ Biomed Pharmacother. 2021;141:111879. https://doi.org/10.1016/j.biopha.2021.111879.
32. Asiwe JN, Kolawole TA, Anachuna KK, Ebuwe EI, Nwogueze BC, Eruotor H, Igbokwe V. Cabbage juice protect against Lead-induced liver and kidney damage in male Wistar rat. Biomarkers. 2022;27(2):151–8. https://doi.org/10.1080/1354X.2021.2022210.
33. Vinson JA, Dabbagh YA, Serry MM, Jang J. Plant flavonoids, especially tea flavonols, are powerful antioxidants using an in vitro oxidation model for heart disease. J Agric Food Chem. 1995;43:2800–2.
34. Bayili RG, Abdoul-Latif F, Kone OH, Diao M, Bassole IH, Dicko MH. Phenolic compounds and antioxidant activities in some fruits and vegetables from Burkina Faso. Afr J Biotech. 2011;10(62):13543–7.
35. Oboh G, Ademosun AO, Akinleye M, Omojokun OS, Boligon AA, Athayde ML. Starch composition, glycemic indices, phenolic constituents, and antioxidative and antidiabetic properties of some common tropical fruits. Journal of Ethnic Foods. 2015;2(2):64–73.
36. Afroz A, Ali L, Karim M, Alramadan MJ, Alam K, Magliano DJ, Billah B. Glycaemic control for people with type 2 diabetes mellitus in Bangladesh-an urgent need for optimization of management plan. Sci Rep. 2019;9(1):1–10.
37. David UE, Asiwe JN, Fasanmade AA. Maternal hypothyroidism prolongs gestation period and impairs glucose tolerance in offspring of Wistar rats. Horm Mol Biol Clin Invest. 2022;43(3):323–8.
38. Al-Badri A, Hashmath Z, Oldland GH, Miller R, Javaid K, Syed AA, Chirinos JA. Poor glycemic control is associated with increased extracellular volume fraction in diabetes. Diabetes Care. 2018;41(9):2019–25.
39. Christensen AS, Viggers L, Hasselström K, Gregersen S. Effect of fruit restriction on glycemic control in patients with type 2 diabetes–a randomized trial. Nutr J. 2013;12(1):1–6.
40. Sadiya A, Mnla R. Impact of food pattern on glycemic control among type 2 diabetic patients: a cross-sectional study in the United Arab Emirates. Diabetes, Metabolic Syndrome Obesity: Targets Therapy. 2019;12:1143–50.
41. Ahmadi S, Awliaei H, Haidarizadeh M, Rostamzadeh J. The effect of ethanolic extract of urtica dioica leaves on high levels of blood glucose and gene expression of glucose transporter 2 (Glut2) in liver of alloxan-induced diabetic mice. Gene Cell Tissue. 2015;2(3):e30355.
42. Ambika S, Saravanan R. Effect of bergenin on hepatic glucose metabolism and insulin signaling in C57BL/6 J mice with high fat-diet induced type 2 diabetes. J Appl Biomed. 2016;14(3):221–7.
43. Okonofua DE, Asiwe JN, Anachuna KK, Moke EG, Sanusi KO, Adagbada EO, Yusuf MO, Alawode DI, Fasanmade AA. Effect of diabetes mellitus and hypertension on osmotic fragility and hemorheological factors in male Wistar rats. Biol, Med Nat Prod Chem. 2021;10(2):73–9.
44. Pantoja PKD, Colmenares DAJ, Isaza MJH. New caffeic acid derivative from Tithonia diversifolia (Hemsl.) A. gray butanolic extract and its antioxidant activity. Food Chem Toxicol. 2017;109:1079–85.
45. Karim N, Rahman A, Chanudom L, Thongsom M, Tangpong J. Mangosteen vinegar rind from Garcinia mangostana prevents high-fat diet and streptozotocin-induced type II diabetes nephropathy and apoptosis. J Food Sci. 2019;84(5):1208–15.
46. Danilova IG, Bulavintceva TS, Gette IF, Medvedeva SY, Emelyanov VV, Abidov MT. Partial recovery from alloxan-induced diabetes by sodium phthalhydrazide in rats. Biomed Pharmacother. 2017;95:103–10.
47. Rashid K, Sinha K, Sil PC. An update on oxidative stress mediated organ pathophysiology. Food Chem Toxicol: Int J Published British Industrial Biol Res Assoc. 2013;62:584–600.
48. Renugadevi J, Prabu SM. Cadmium-induced hepatotoxicity in rats and the protective effect of naringenin. Exp Toxicol Pathol. 2010;62(2):171–81.
49. Nasiri A, Ziamajidi N, Abbasalipourkabir R, Goodarzi MT, Saidijam M, Behrouj H, et al. Beneficial effect of aqueous garlic extract on inflammation and oxidative stress status in the kidneys of type 1 diabetic rats. Indian J Clin Biochem. 2017;32:329–36.
50. Mobasher MA, Germoush MO, Galal El-Tantawi H, Samy El-Said K. Metformin improves biochemical and pathophysiological changes in hepatocellular carcinoma with pre-existed diabetes mellitus rats. Pathogens. 2021;10(1):59.