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Table of Content: Volume 9 Number 3 September 2018

ARTICLE

Antidiabetic and anti- hyperlipidemic effects of ethanolic extract of Dryopteris dilatata leaves

20

Akpotu Ajirioghene, Celestine Ani, Choice Nworgu, Okorie Pamela, Igwe Uzoma, Jide Uzoigwe, Adeyemo Mercy, Nwaeme Ogochukwu and Obinna Onwujekwe

Full Length Research Paper

Antidiabetic and anti- hyperlipidemic effects of ethanolic extract of *Dryopteris dilatata* leaves

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The incidence of diabetes mellitus (DM) is increasing globally and it is a major source of concern. This study was undertaken to assess the antidiabetic effect of the ethanolic leaf extract of *Dryopteris dilatata* (ELEDD). Thirty adult Wistar rats with body weight (BW) of 120-150 g were randomly assigned to groups of five rats each (n=5). Groups 1 served as normal control; Groups 2-6 were diabetic groups; group 2 served as negative control; group 3 received 50 mg/kg of metformin; 4-6 received 200, 400 and 800 mg/kg of ELEDD respectively. The BW and fasting blood glucose level (FBSL) of the rats were monitored weekly. At the end of the experiment, all the rats were anaesthetized with 25% urethane (sigma- Aldrich) intraperitoneally (I.P) and blood samples were collected by cardiac puncture for biochemical analysis. There was an increase in the BW of the metformin treated group and varying doses of ELEDD. It caused 77.00±15.07% decrease in FBSL; 86.94±34.80% and 248.07±20.56% with respect to 400 and 800 mg/kg of ELEDD. There was a significant (p<0.05) increase in the serum total cholesterol (STC), triglycerides (TG) and low density lipoprotein (LDL-C) as well as decrease in high density lipoproteins (HDL-C). Lipid profile groups treated with ELEDD significantly (p<0.05) decreased in a dose dependent manner. This study has shown that DD has hypoglycemic and hypolipidemic effects.

Key words: Diabetes, *Dryopteris dilatata*, hypoglycaemic, hypolipidaemic and phytochemical

INTRODUCTION

Diabetes mellitus (DM) is a group of metabolic disorder, characterized by hyperglycemia with disturbance in

carbohydrates, fats and protein metabolism. It results from either defect in insulin secretion by the pancreas

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secretion by the pancreas or inability of the cells to utilize the insulin produced (Shoback et al., 2011). Insulin is secreted by the beta-cells of the islet of Langerhans in the pancreas. Diabetes is a prime risk factor for cardiovascular disease. Cardiovascular disorders include peripheral vascular disease, stroke, and coronary artery disease. Diabetes also affects the heart muscle causing both systolic and diastolic heart failure and also includes polyuria, polyphagia, polydipsia, and ketosis (Thrains et al., 2005). Life for a person with diabetes mellitus means constant awareness of the illness, one or two insulin shots a day, frequent finger punctures to monitor blood glucose level, a restrictive diet, and concern over complications. Global diabetes prevalence has more than doubled over the last three decades, with prevalence rates far exceeding modeled projections; even after allowing for improved surveillance. Nearly 1 in 10 adults worldwide are now affected with diabetes (Danaei et al., 2011). This striking statistic has led to investigation into the population divers of diabetes prevalence. Presently, there is renewed interest in the use of herbal products. This may be attributable to the down turn in the economy, as herbal medicine is perceived to be a cheaper means of treatment (Acuff et al., 2004). The World Health Organization estimated that over 80% of the people in developing countries rely on traditional remedies such as herbs for their daily needs and about 855 traditional medicines include crude plant extracts (Tripathi et al., 2003). DM is one of the world's leading causes of death, with over 150 million diabetic cases worldwide (Danaei et al., 2011). More than 1.70 million Nigerians above 15 years old are diabetic, with about 70,000 children less than 15 years developing Type 1 diabetes annually. Diabetes is prevalently rising globally as a result of obesity, population growth and sedentary lifestyles, and it is projected to be over 360 million cases by 2030 (Wild et al., 2004). Diabetes is an incurable endocrine disorder, but efforts have been made through the use of oral hypoglycaemic agents (sulphonylureas) for its management to prevent life-threatening complications that might arise. Cost, undesirable adverse effects associated with these drugs promote the use of suitable herbs with hypoglycemic activities. Over 50% of plants serve in traditional medicine for their health benefits in combating certain ailments affecting humans such as dysentery, diarrhea, toothache, skin infections and diabetes. One of such plant considered as having great importance is DD. Many conventional drugs have been derived from prototypic molecules in medicinal plants. Metformin exemplifies an efficacious oral glucose-lowering agent. To date, over 400 traditional plant treatments for diabetes have been reported, although only few of the plants have received scientific and medical evaluation to assess their efficacy (Dokken, 2008). Based on the WHO recommendations, hypoglycemic agents of plant origin used in traditional

medicine are important for the control of blood glucose (WHO, 1980). The attributed hypoglycemic effects of these plants is due to their ability to restore the function of pancreatic tissues by causing an increase in insulin output or inhibit the intestinal absorption of glucose or to the facilitation of metabolites in insulin dependent processes. Hence, treatment with herbal drugs has an effect on protecting β -cells and smoothing out fluctuation in glucose levels. Most of these plants have been found to have chemical constituents like glycosides, alkaloids, phenols, terpenoid and flavonoids that are frequently connected as having antidiabetic effects, (Loew and Kaszkin, 2002). One of such plant considered with great importance is DD. DD (*Broad buckler fern*) is a medicinal plant belonging to Dryopteridaceae family. It grows to 120 cm tall and 90cm wide, with dark green tripinnate fronds, with the ribs covered in brown scales, (Runk et al., 2012). It is known as Okpomie in Olomoro in Isoko South Local Government Area, Delta State, Nigeria. DD is distributed throughout tropical region of Nigeria and people from Olomoro in Isoko South Local Government Area of Delta State make concoctions of this plant when diabetic and their conditions appear better. The leaves and roots are used for medicinal purposes. It is also commonly used as an Anti-dandruff and worm expeller (Brown et al., 2011). Progressive insulin resistance is mainly accompanied with pro-atherogenic cardiovascular risk profiles and consequently atherosclerotic coronary artery disease and other forms of cardiovascular disease are the major causes of mortality in type 2 diabetic patients (Kalofoutis et al., 2007). Hyperlipidemia, undesirable changes in vascular endothelial and smooth muscle cells, lipid peroxidation especially oxidized low-density lipoprotein particles, oxidative damage and increased inflammatory mediators including chemokine's and cytokines, hyper-coagulation and platelet activation are considered as the main metabolic abnormalities in diabetes mellitus leading to cardiovascular disease (Thomas et al., 2007). There is growing evidence suggesting that the use of polyphenol-rich plants with these bioactive components could have protective effects against diabetes-induced cardiovascular pathogenesis; the mechanisms involved in these properties mainly include regulation of lipid metabolism, attenuation of oxidative damage and scavenging of free radicals, improvement of the endothelial function and vascular tone, enhancing the production of vasodilation factors such as nitric oxide, and inhibiting the synthesis of vasoconstrictors such as endothelin-1 in endothelial cells (Stoclet et al., 2004; Schini-Kerth et al., 2010; Lecour et al., 2011). Urbanization and its effects on the life style of individuals, especially obesity, have increased the prevalence of DM in Nigeria according to the estimates of the Diabetic Association of Nigeria (DAN).

DM is a global burden, associated with life-threatening complications including stroke, renal failure and cardiac

attack. The existing management of diabetes using orthodox and various medications is expensive, it requires prolong use, inadequate storage facility in rural areas and accompanied with several side effects that have reduce compliance. Herbal remedies are easily accessed and inexpensive.

The rise in glucose level on induction of diabetes results to a corresponding increase in serum lipids. The significant increase in the level of serum lipids in diabetes can be due to the lack of insulin, since under normal condition, insulin activates the enzyme lipoprotein lipase and hydrolyzes serum lipids. However, in diabetic state lipoprotein lipase is not activated due to insulin deficiency resulting in hyperlipidemia (Akah et al., 2004; Nwanjo et al., 2006; Ayinla et al., 2011). It is with all this background that this study is thus aimed at investigating the anti-diabetic effect of ELEDD and its associated hyperlipidemia on alloxan induced diabetic Wistar rats.

MATERIALS AND METHODS

Collection of the *D. dilatata* plants

The plant was collected from a wide growing habitat in Olomoro Community in Isoko South Local Government Area of Delta State. A plant specimen was authenticated by a taxonomist (Dr. Harrison Erhenhi) in the Department of Botany, Delta State University Abraka with a voucher specimen number FHI 1100338.

Preparation of ethanolic leaf extract of *D. dilatata*

The ELEDD was prepared according to the method of Wycliffe et al. (2016). The leaves of DD were collected and washed to remove dust particles and other microbial organisms that might adhere to the surface of the leaves. They were air dried at room temperature ($25 \pm 1.02^\circ\text{C}$) after which they were milled into fine powder using a Century electric blender (CB- 8283-J), a product of Century Company Ltd. Oaks, California; and sieved to fine powder with porcelain cloth. 300 g was weighed into a beaker and macerated in 1500 ml of ethanol followed by shaking at 2 h intervals for 72 h and was then filtered with No. 1 Whatman filter paper (110 × 100 mm) to obtain a filtrate which was further evaporated in the hot air oven (Drawell Scientific Ltd; Model; DGT-G25. The granules were weighed and refrigerated at -2°C in an air tight container till ready for use.

Phytochemical screening method

The quantitative and qualitative phytochemical analysis was done using standard methods (Chukwuma et al., 2016).

Experimental procedure

The experiment was carried out at the Animal House Unit, Faculty of Basic Medical Sciences, Delta State University Abraka (DELSU). The Animals were handled in accordance with the guidelines and principles of the University Ethical Committee. Animals were fed with rat chow from Flour Mills Nig Ltd, Sapele, and distilled water

under hygienic conditions and maintained in normal and standard laboratory condition with 12hrs light/dark cycle with adequate ventilation for the duration of the experiment.

Acute toxicity test (LD₅₀)

Lethal dose (LD₅₀) was investigated according to the method of Lorkes (1983). The wistar rats were divided into two phases; each phase contains three groups of four rats each. ELEDD was administered orally in divided doses to six different groups of four rats each using oral cannula.

First phase

Group 1: Received 600 mg/kg orally

Group 2: Received 1200 mg/kg orally

Group 3: Received 1800 mg/kg orally

Second phase

Group 1: Received 2400 mg/kg orally

Group 2: Received 3000 mg/kg orally

Group 3: Received 3600 mg/kg orally

All the rats were monitored for mortality and morbidity and for other signs of toxicity for 24 h. Food was withheld from the rats for 12 h, but they were allowed access to drinking water *ad libitum*. At the end of the observation, no obvious signs of toxicity in all the treatment doses after the administration of the extract to the experimental animals. All the animals survived after twenty four (24 h) of observation. The LD₅₀ at a higher dose 3600 mg/kg did not cause any toxic effect on the Wistar rat. This result shows that the ELEDD has a high margin of safety. FBSL were checked after this period using an ACCU-ANSWER (zh-g01) glucometer (Guillin Royalze Medical Instrument Co. Ltd China) and blood was gotten from the caudal vein of the rats. The rats with glucose levels of 85 mg/dl (Wang et al., 2014) were considered normoglycemic and used for the study.

Drug preparation and administration

Alloxan monohydrate solution was prepared using the method of Wycliffe et al. (2016). 100 mg of powered Alloxan obtained from Sigma Aldrich (Steinhen, Switzerland) was dissolved in 1 ml normal saline as vehicle to get the appropriate concentration that was administered to the rats after overnight fast (8 to 12 h). The rats were induced with diabetes by the administration of a single intraperitoneal injection of the dissolved alloxan monohydrate at a dose of 150 mg/kg body weight (Nayeemunnisa, 2009). Ten tablets of metformin of 5 mg each were dissolved in 100 ml of normal saline to get the appropriate concentration of 1 mg/2 ml that was administered to the rats. Thereafter, the rats were fed with normal feed and water. Two days (48 h) after induction, diabetes was confirmed with a fasting blood glucose level of $\geq 200\text{mg/dl}$, using the ACCU-ANSWER glucometer (Szkudski, 2001).

EXPERIMENTAL DESIGN

Group 1: Normal control and received water and feed *ad libitum*.

Group 2: Diabetic untreated group (Negative control)

Group 3: Diabetic and received 50 mg/kg metformin as standard diabetic drug

Group 4: Diabetic and received 200 mg/kg ELEDD

Group 5: Diabetic and received 400 mg/kg ELEDD

Group 6: Diabetic rats and received 800 mg/kg ELEDD

Method of In vivo fasting blood sugar measurement

Fasting blood sugar level assay was done according to the method of Wycliffe et al. (2016). The blood was collected by gently "milking" the tail from the body towards the tip after sterilization with 70% ethanol, the nipping with a sharp scissors to initiate bleeding and blood glucose levels were determined weekly for four weeks.

Blood collection for analysis of lipid profile

Blood for the analysis of the lipid profile was collected using the method of Ibegbulem et al. (2012). At the end of the experiment, the animals fasted overnight and the rats were anesthetized with 25% urethane and blood was collected by cardiac puncture, using 5 ml syringes and 21G needles into a plain blood sample containers and allowed to clot. The blood samples were centrifuged at a rate of 4000 rpm for 10 min using the Boeltz Desktop centrifuge New Delhi India and the serum was collected and stored in the refrigerator at 4°C for analysis. Stored serum sample were analyzed for HDL-C, TG, and TC concentrations as determined by enzymatic determination, using the kits purchased from Randox laboratories Ltd, United Kingdom. LDL was calculated from the Friedwald formula (LDL= [Total cholesterol – HDL – TG/5] mg/dl).

Determination of body weight

Body weight of experimental rats was determined according to the method of Ani et al. (2017) using a top loading weighing balance by Havard apparatus USA at week 0 (before administration) and subsequent weeks till the last day of experiment. Percentage weight gain was later calculated as follows:

$$\text{Percentage weight gain} = \frac{\text{Initial weight} - \text{final body weight}}{\text{Initial body weight}} \times 100$$

Blood glucose levels of experimental animals were determined at week 0 (before administration) and subsequent weeks and the last day of experiment. Percentage change in glucose level was later calculated as follows:

$$\text{Percentage change in glucose level} = \frac{\text{Initial-final glucose level}}{\text{Initial glucose level}} \times 100$$

Lipid Profile Analysis

The lipid profile analysis was done according to the method of Ibegbulem et al. (2012). The procedure involved pipetting 1000ul of the reagents into three test tubes labeled Standard (S), Test (T) and Blank (B) followed by 50 ul of the reagents and samples at room temperature, into labelled tubes, mixed and the tubes were allowed to stand for fifteen (15) minutes in an incubator at 25°C or five (5) minutes at 37°C. Then, the absorbance (A) of the samples and the standard were read on the spectrophotometer at 550nm against the blank reagent (24, 25). The calculation of the total cholesterol is gotten from the relationship:

$$\frac{A \text{ sample}}{A \text{ Standard}} \times \frac{\text{concentration of the standard}}{1} = \text{mg/dl of STC}$$

High density lipoproteins (HDL)-cholesterol measurement is based on the principle of a separation method, using the selective precipitation of apolipoprotein B-containing lipoproteins (VLDL, LDL and (a) Lpa) by phosphotungstic acid/MgCl₂, sedimentation of the precipitant by centrifugation, and subsequent enzymatic analysis of high density lipoproteins (HDL) as residual cholesterol remaining in the clear supernatant. The procedure involved pipetting equal amounts of the reagents and samples at room temperature into labelled centrifuged tubes vortex; and allowed to stand for ten (10) minutes at room temperature after which they were centrifuged for ten (10) minutes at revolution per minute then the clear supernatant was separated within two (2) hours (24, 26). The calculation of the total high density lipoprotein is gotten from the relationship:

$$\frac{\text{Absorbance of supernatant}}{\text{Absorbance of Standard}} \times \text{Concentration of standard} = \text{Mg/dl of HDL-C}$$

Triglycerides based on the enzymatic hydrolysis of serum to triglycerol and free fatty acids (FFA) by lipoprotein lipase (LPL). The glycerol is phosphorylated by adenosine triphosphate (ATP) in the presence of glycerolkinase (GK) to form glycerol 3 phosphate (G3P) and adenosine diphosphate (ADP). G 3 P is oxidized by glycerol phosphate oxidase (G P O) to form dihydroxyacetone phosphate (DHAP) and hydrogen peroxide.

$$\frac{A \text{ supernatant}}{A \text{ Standard}} \times \text{Concentration of standard} = \text{mg/dl HDL-C}$$

The procedure involved pipetting the reagent and sample at room temperature into labeled tubes, mixed and the tubes were allowed to stand for 15 min at room temperature. The absorbance (A) of the samples and standard were read at 500 nm against the reagent blank (Eddouks et al., 2002). The calculation of the total triglyceride is gotten from the relationship.

Statistical analysis

The data were analyzed using statistical package for social sciences (SPSS) version 21. Results were expressed as Mean ± SEM (standard Error of Mean) and one way analysis of variance (ANOVA), followed by post Hoc Fisher's test for multiple comparison. P < 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Table 1 shows the quantitative and qualitative screening of the ELEDD which revealed the presence of phytochemical such as phenols, phytosterols, tannins, triterpenoids, flavonoids, saponins, glycosides, steroids, terpenoids, and alkaloids cardiac glycosides; while anthraquinones and phlobotanines were absent. Literatures from previous studies confirm the presence of tannins, alkaloids, glycosides and saponins in this class of plants (Singh et al., 2007). This could be used as an acid test to attest to the fact that the indigenes could also

Table 1. Qualitative and quantitative result of the phytochemical screening of the ethanolic extract of *D. dilatata*.

Phytochemicals	Qualitatives	Quantitatives (%)
Alkaloids	++	6
Saponins	++	13
Tannins	++	7.8
Flavonoids	+	5
Phenols	+++	9.7
Reducing sugar	---	0.16
Total carbohydrate	++	0.20
Steroids	+ --	
Terpenoids	+--	
Phytosterols	++	
Triterpenoids	+++	
Phlebatannins	--	

Keys: + + + abundantly present, + + - Moderately present, + - - Present in trace amount, --- Absent.

Table 2. Effect of ethanolic leaves extract of *Dryopteris dilatata* on the body weight (gram) of the experimental animals.

Groups	Week 0	Week 1	Week 2	Week 3	Week 4
1	140.40±3.70	140.56±3.59	143.02±2.39	147.18±2.24	150.94±2.95
2	144.56±3.58	133.58±2.52	129.26±2.76	124.36±3.17	118.44±3.00
3	147.42±1.77	140.50±.82	141.96±2.10	131.94±2.25	135.96±2.73
4	146.04±1.88	133.20±2.03	137.58±3.15	137.70±2.09	140.02±2.70
5	145.44±2.03	131.78±1.04	129.74±3.24	132.90±3.32	137.90±3.36
6	139.20±3.20	131.78±2.47	132.10±2.97	137.44±2.91	142.46±3.08

Values are expressed as Mean±SEM. ANOVA followed by Tukey's post-hoc test for multiple comparison.

use this plant for other ailments such as antiviral, anti-allergic, anti-inflammatory and anti-oxidant agent. In the acute toxicity test, there was neither death recorded nor discernible effect in any of the rats dosed with 600, 1200, 1800, 2400, 3000 and 3600 mg/kg of the ELED; thus 3600 mg/kg was considered as safe for administration.

The median lethal dose (LD₅₀) could not be determined as the plant extract showed a high margin of safety after receiving 3600 mg/kg of ELED. According to Clarke and Clarke (1979), any substance whose LD₅₀ is above 1000 mg/kg is regarded relatively safe. The change in body weight of positive control and hyperglycemic rats is at day 0 before induction of alloxan monohydrate and Day 1 after induction to week 1, 2, 3 and 4 of administration. The administration of metformin (50 mg/kg), and ethanol extract of *Dryopteris dilatata* (200, 400 and 800 mg/kg) on hyperglycemic rats was not significant at week 1 and 2 but was significant at week 3 at (P < 0.05) with negative control as compared to positive control (Table 2). Table 3 shows the percentage of changes in body weight of experimental animals between initial and final body

weight and baseline, and final body weight of all groups. The blood glucose level of both positive control and hyperglycemic rats at day 0 before induction of alloxan monohydrate, and day 1 after induction to week 2, 3 and 4 of administration were observed. The administration of metformin (50 mg/kg), and ELED (200, 400 and 800 mg/kg) on hyperglycemic rats was not significant (P>0.05) at week 2 to 3, while it was significant at week 4 (P < 0.05) with negative control as compared to positive control. In the case of the diabetic rats, while treatment with metformin caused an increase in body weights of the rats, treatment with the three doses of ELED caused mild increase of body weights, indicating that the mechanisms involved in the hypoglycemic effect of metformin (50 mg/kg), and ELED (200, 400 and 800 mg/kg) may be similar.

Table 4 shows the Percentage changes in glucose level of the experimental animals between initial and baseline, initial and final, as well as baseline and final fasting glucose level of the experimental animals of all groups. Metformin has been shown to increase the

Table 3. Percentage changes in the body weight of the experimental animals.

FBS groups	% Δ in initial and final bw	% Δ in baseline and final bw
1	0.4 \pm 1.32 ^b	7.39 \pm 1.27 ^b
2	-22.21 \pm 3.11 ^a	-12.87 \pm 1.17 ^a
3	-8.64 \pm 2.82 ^b	-3.47 \pm 1.73 ^b
4	-4.43 \pm 2.09 ^b	4.74 \pm 2.30 ^b
5	-5.83 \pm 3.83 ^b	4.28 \pm 1.70 ^b
6	2.25 \pm 1.59 ^b	7.45 \pm 1.11 ^b

Values are expressed as mean \pm SEM. ANOVA followed by Tukey Post-hoc test multiple test. Values not sharing a common superscript differ significantly at P<0.05. % Δ = percentage change; FBS= Fasting blood sugar.

Table 4. Effect of ethanol leaves extract of *D. dilatata* on glucose level (mg/dl) of the experimental animals.

Groups	Week 0	Week 1	Week 2	Week 3	Week 4
1	84.40 \pm 6.82	87.00 \pm 2.05	91.00 \pm 3.63	93.40 \pm 4.52	97.00 \pm 3.85
2	85.00 \pm 3.65	298.60 \pm 38.85	321.60 \pm 30.64	352.80 \pm 33.94	600.00 \pm .00
3	87.20 \pm 3.92	317.40 \pm 12.53	227.80 \pm 19.29	205.80 \pm 8.99	124.00 \pm 4.60
4	91.20 \pm 2.78	295.00 \pm 30.98	279.20 \pm 30.15	233.20 \pm 4.99	166.20 \pm 7.00
5	79.20 \pm 5.20	303.60 \pm 54.72	246.00 \pm 29.52	199.80 \pm 14.42	161.40 \pm 12.19
6	88.60 \pm 3.01	402.80 \pm 30.93	291.80 \pm 38.32	182.00 \pm 20.52	115.80 \pm 5.40

Values are expressed as Mean \pm SEM. ANOVA followed by Tukey Post-hoc test for multiple comparison.

sensitivity of peripheral tissues to insulin; thereby increasing glucose uptake by the tissues that is translocation of glucose transporters in muscle and adipose tissue to increase their glucose uptake (Ojeh et al., 2015) and the inhibition of free fatty acids release into the circulation due to suppression of the activity of hormone-sensitive lipase and a simultaneous increase in their clearance from the circulation (Mayfield, 1998).

In the present study, metformin (50 mg/kg), and ELEDD (200, 400 and 800 mg/kg) induced a significant decrease in FBSL of diabetic Wistar rats as compared to the negative control. In this context, the number of other insulin stimulatory effects (Salah et al., 1995). The significant increase in blood glucose level on treatment with alloxan monohydrate shows that diabetes was induced in the rats. The diabetic effect of alloxan monohydrate has been attributed to a specific cytotoxic plants has also been reported to have anti-diabetic and action mediated by hydroxyl radical generation in pancreatic β -cell, which damages a large number of β -cells resulting in a decrease in endogenous insulin release (Szkudski et al., 2001). These result in elevated blood glucose within a short period of time after alloxan monohydrate administration as it had been highlighted by others (Haidari et al., 2012). In the present study, it was observed that ELEDD 200, 400 and 800 mg/kg reversed from 295-166, 303-161 and 402-115 mg/dl respectively.

The results were in consonance with reports by Asuquo

et al. (2010) and Okokon et al. (2013) who demonstrated the hypoglycemic effect of tested extract on wistar rats. Although the mechanism of action of the extract was not explored, studies have shown that the causes and sites of intervention in the biochemical process of diabetes is diverse (Larner, 1985). One of such is that blood glucose lowering drugs may act through stimulation of synthesis and/or release of insulin from the beta-cells of the pancreatic islets, which may also increase sensitivity of receptors to insulin inhibiting effect, and stimulation of peripheral tissues uptake of glucose cannot be ruled out (Szkudski et al., 2001). Also, it had been reported that some herbs or plants reduce absorption of carbohydrates in the digestive system, causing progressive entry of glucose into the blood and prevent sudden increase in blood glucose after food intake (Jenkins et al., 1980). The significant increase in the level of triglyceride of diabetic control rats found in this study might be due to lack of insulin. Since under normal condition, insulin activates the enzyme lipoprotein lipase and hydrolyzes triglyceride. However, in diabetic state, lipoprotein lipase is not activated due to insulin deficiency resulting in hypertriglyceridemia. Metformin (50 mg/kg) and ELEDD (200, 400 and 800 mg/kg) reduced triglycerides in the serum of alloxan-induced diabetic rats and might prevent the progression of coronary heart disease. They might inhibit endogenous glucose production or interfere with gastrointestinal glucose absorption (Eddouks et al., 2002)

Table 5. Percentage changes (Δ %) in fasting glucose level of the experimental animals

FBS groups	% Δ in initial and baseline FGL	% Δ in initial and final FGL	% Δ in baseline and final FGL
1	2.89 \pm 7.79 ^a	13.05 \pm 6.00 ^a	10.04 \pm 2.01 ^a
2	69.30 \pm 4.49*	85.83 \pm 0.61*	50.23 \pm 6.48*
3	72.40 \pm 1.50 ^a	28.96 \pm 5.44 ^a	-157.02 \pm 11.76 ^a
4	67.57 \pm 3.64 ^a	44.58 \pm 3.49 ^a	-77.00 \pm 15.07 ^a
5	68.54 \pm 7.98 ^a	50.05 \pm 3.90 ^a	-86.94 \pm 34.80 ^a
6	77.43 \pm 2.04 ^a	22.52 \pm 5.62 ^a	248.07 \pm 20.56 ^a

Values are expressed as Mean \pm SEM. ANOVA followed by Tukey post-hoc test for multiple comparison. Values not sharing a common superscript differ significantly at $P < 0.05$. % Δ = percentage change; FBS = Fasting blood sugar.

Table 6. Effect of ethanolic leaf extract of *dryopteris dilatata* on lipid profile (mg/dl) diabetic rats.

Groups	STC	TG	HDL	LDL
1	138.06 \pm 6.09 ^a	156.82 \pm 11.21 ^a	49.32 \pm 5.08 ^a	128.18 \pm 4.14 ^a
2	191.38 \pm 12.48 ^b	359.37 \pm 107.4 ^b	22.03 \pm 5.54 ^b	190.10 \pm 9.58 ^b
3	157.02 \pm 9.43 ^a	219.98 \pm 7.22 ^d	42.37 \pm 4.62 ^a	136.11 \pm 7.43 ^a
4	171.94 \pm 10.83 ^d	204.73 \pm 44.56 ^d	35.70 \pm 3.90 ^d	151.65 \pm 7.87 ^c
5	140.55 \pm 12.97 ^a	162.98 \pm 12.74 ^a	41.80 \pm 1.10 ^a	138.34 \pm 1.45 ^a
6	112.84 \pm 3.33 ^c	123.09 \pm 2.24 ^c	50.16 \pm 5.30 ^a	137.44 \pm 6.81 ^a

Values are expressed as mean \pm SEM. ANOVA followed by LSD's multiple range tests. Values not sharing a common superscript differ significantly at $P < 0.05$.

(Table 5).

There was a significant increase in the STC, TG and LDL in diabetic rats as compared to positive control. The administration of metformin (50 mg/kg) and ELEDD (200, 400 and 800 mg/kg) brought back the levels of STC, TG and LDL to normal and there was a significant decrease in the HDL-C in diabetic rats as compared to positive control. The administration of metformin (50 mg/kg) and ELEDD (200, 400 and 800 mg/kg) brought back the levels of HDL-C to normal. The low concentration of HDL and high concentration of LDL observed in diabetic rats compared to negative control are consistent with reports from several studies (Akah et al., 2004; Nwanjo et al., 2006v; Ayinla et al., 2011) demonstrating that a rise in glucose level on induction of diabetes, might result in a corresponding increase in serum lipids. It has been reported that elevated serum lipids in diabetes is due to the increased mobilization of free fatty acids from peripheral fat depots as a result of inhibition of the hormone sensitive lipase Ekeocha et al., (2012) (Table 6).

The excess fatty acids produced are converted into phospholipids and cholesterol, which together with excess triacylglycerols formed at the same time in the liver are discharged into the blood in form of lipoproteins. Thus, the marked hyperlipidemia observed in diabetic rats might be regarded as a consequence of inhibited

actions of lipolytic hormones in fats depots (Goodman and Gilman, 1985). Treatment of diabetic rats with ELEDD caused a significant decrease in serum of cholesterol (191-112 mg/dl), triglyceride (359-123 mg/dl) and low density lipoprotein (190-137 mg/dl) when negative control is compared with 800mg/kg of ELEDD, showing its hypolipidemic effect. The results of this study support earlier reports that hypolipidemic effect of plants may be related to its active ingredients (Akah et al., 2004; Nwanjo et al., 2006v; Mayfield, 1998; Ayinla et al., 2011: 20) and this could be related to the presence of alkaloids, saponins, flavonoids and polyphenols (Ayoola et al., 2009) known to reduce serum lipid level in wistar rats (Okokon et al., 2013).

Conclusion

This study has shown that *D. dilatata* has hypoglycaemic and hypolipidaemic effects in diabetic state, and therefore, has the ability to ameliorate possible complications associated with diabetes mellitus, such as atherosclerosis diseases.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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