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Antidiabetic and Antidyslipidemic Activities of *Dryopteris Filix-Mas* Methanol Leaf Extract in Alloxan-Induced Diabetic Rats

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ABSTRACT

Diabetes mellitus, a chronic metabolic disease with significant morbidity and mortality, has been associated with changes in lipid profile. *Dryopteris filix-mas* has been widely explored in treatment and management of various disorders. The antidiabetic and antidyslipidemic effects of *Dryopteris filix-mas* in alloxan-induced diabetic rats were investigated in Wistar rats. Thirty (30) adults male Wistar rats used were grouped into: 1 (Normal control); 2 (Untreated alloxan induced-diabetic rats); 3 (Diabetic rats administered 500mg/kg metformin); 4 (Diabetic rats administered 100mg/kg *Dryopteris filix* methanol leaf extract). 5 (Diabetic rats administered 250mg/kg *Dryopteris filix* methanol leaf extract); 6 (Diabetic rats administered 500 mg/kg *Dryopteris filix* methanol leaf extract). The rats were sacrificed after 21 days. The serum was used for biochemical assay of glucose, cholesterol, triglyceride, high density lipoprotein (HDL) and low-density lipoprotein (LDL), using standard procedures. *Dryopteris filix* leaf extract significantly (p<0.05) reduced blood glucose levels in rats after initial increase in glucose following diabetes induction. Induction of diabetes in rats significantly (p<0.05) increased serum cholesterol, triglyceride and LDL levels, while significantly reducing HDL levels in diabetic rats. However, administration of varying doses of *Dryopteris filix-mas* extract significantly (p<0.05) decreased levels of cholesterol, triglyceride and LDL with corresponding increase in level of HDL. The observed changes compared well with the group administered metformin. The methanol leaf extract of *Dryopteris filix-mas*, thus, improved serum lipid profile, in diabetic rats, along with having antihyperglycemic activity.

Keywords: Diabetes, Lipid profile, *Dryopteris filix-mas*, Antidiabetic activity, Antidyslipidemic activity.

Introduction

Diabetes Mellitus is a group of metabolic diseases characterized by increased blood glucose level resulting from deficiency in insulin action, insulin secretion, or both.[1] The disease prevalence is on the increase in developing nations. Studies showed that for some 35 years period, the disease prevalence among adult men increased from 4.3 percent to 9.0 percent while it also increased from 5.0 percent to 7.9 percent among adult women.[2] Diabetes remains a significant global health burden. Patients with type-2 diabetes have higher risk of cardiovascular disease associated with atherogenic dyslipidemia.[3] Prevalence of dyslipidemia in diabetes mellitus is significantly high[4] Dyslipidemia is a significant risk factor for coronary heart disease (CHD). Cardiovascular disease is a cause of morbidity and mortality in diabetes mellitus patients because of disturbance in lipoproteins such as High-Density Lipoprotein cholesterol (HDL), serum cholesterol, serum triglycerides (TC), and Low-Density Lipoprotein cholesterol (LDL).[4] There is an increasing demand by patients to use natural products with antidiabetic activity. The utilization of ethnomedicinal plants in treating various illnesses can be traced to as far back as ancient history.[5]

Herbs have always formed an integral part of human health and are used in treating several human diseases.

One of such herbal species that is broadly employed in traditional medicine is *Dryopteris filix-mas. Dryopteris filix-mas* is being used to cure splenomegaly in malaria infection, feverish condition and dropsy.[6] In Nigeria, it is utilized in various parts of Edo and Delta State, in treating gastrointestinal disorders, management of rheumatic disorders, boils and sores. The cooked young frond is eating as an aid in losing weight.[7] Furthermore, the plant has been shown to possess potent antioxidant activity,[8] anti-inflammatory activity,[9] and wound-healing activity.[10] This study was aimed at investigating the antidiabetic and antidyslipidemic activity of *Dryopteris filix-mas* in alloxan-induced diabetic rats.

Materials and Methods

Plant materials

Fresh whole plants of *Dryopteris filix-mas* were harvested from an uncultivated farmland in Abraka, Ethiope East Local Government Area of Delta State, Nigeria in March, 2021. The plants were authenticated by a Botanist at Botany Department, Delta State University, Abraka, Nigeria (voucher number – 1013584).

Animals

Adults male Wistar rats (30) weighing between 100-188 g were used. They were maintained at Emma-maria Scientific Research Laboratories and Consultancy, Abraka, Nigeria. The Wistar rats were kept in plastic cages under controlled condition of 12hrs light / 12hrs dark cycle and allowed access to standard rat feed and water *ad libitum*. The maintenance of the animals was in accordance with

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approved guidelines by the Animal Ethics Committee, Delta State University, Abraka, Nigeria (ethical approval number - REC/FBMS/DELSU/2021/139).

Extract preparation
The leaves were plucked, washed, air-dried and blended to produce a fine powder (200 g) which was macerated in 1000 mL of methanol and allowed to stand for 48 hours before filtering. Thereafter, the extract was concentrated to form a gel-like liquid using cylindrical burner at 40°C. The percentage yielded was recorded.

Induction of diabetes in animals
Diabetes was induced in the experimental animals by a single intraperitoneal injection of 150 mg/kg alloxan monohydrate. Then 5% dextrose was administrated to combat the immediate hypoglycemia. Two days after alloxan injection, glucose levels in the rats were determined using a glucometer, and rats with levels of greater than 140 mg/dL were included in the study. Treatment of the experimental rats with *Dryopteris filix-mas* extracts was started 48 hours after injection of alloxan.

Experimental design
The rats were allotted into six groups with five (5) rats each as follows:
Group 1: Normal control group.
Group 2: Untreated Diabetic rats (negative control)
Group 3: Diabetic rats administered 500 mg/kg of metformin.
Group 4: Diabetic rats administered 100 mg/kg of *Dryopteris filix* methanol leaf extract.
Group 5: Diabetic rats administered 250 mg/kg of *Dryopteris filix* methanol leaf extract.
Group 6: Diabetic rats administered 500 mg/kg of *Dryopteris filix* methanol leaf extract.
The extracts were administered orally daily for 21 days. The weights of the rats were checked every seven (7) days, and their glucose levels every five (5) days during the 21-days period.

Animal sacrifice and sample collection
At the end of the specified period of administration, rats were sacrificed by cervical decapitation after an overnight fast. After sacrifice, blood samples of the rats were immediately collected by cardiac puncture using a hypodermic syringe and needle. The blood collected was transferred into collection tubes and spun in a centrifuge at 4000g for 10 min. Serum was obtained from the already separated blood and placed in the refrigerator for storage until further biochemical analysis.

Biochemical analysis
The biochemical investigations were carried out on the obtained serum to determine lipid profile (high-Density Lipoprotein, low density lipoprotein, triglyceride and cholesterol) of the sacrificed rats using standard procedures.

Total Cholesterol
Total cholesterol was determined according to method previously described by Allain *et al*.[11] 2.0 mL of cholesterol reagent was added to test tubes labelled blank, standard and samples (in triplicates). 20µL of cholesterol standard was added to test tube labelled standard, while 20 µL of the serum was added to the sample test tubes. The test tubes were then incubated for 10 min at 37°C and absorbance measured at 500nm.

$$\text{Cholesterol Conc. (mg/dL)} = \frac{Absorbance\ of\ test\ sample}{Absorbance\ of\ standard} \times \text{Concentration of std (mg/dL)}$$

HDL
HDL-Cholesterol level in serum was determined according to method previously described by Castelli *et al*.[12] 1.0 mL of HDL-cholesterol reagent was added to test tubes labelled blank, standard and samples (in triplicates). 50 µL of distilled water was added to blank, 50 µL of cholesterol standard was added to test tube labelled standard, while 50 µL of the serum was added to the sample test tubes. The test tubes were then incubated for a period of 10 min at 37°C and absorbance measured at 500 nm.

$$\text{HDL Conc. (mg/dL)} = \frac{Absorbance\ of\ test\ sample}{Absorbance\ of\ standard} \times \text{Concentration of std (mg/dL)}$$

Triglycerides
Triglyceride level in the serum was determined according to method previously described by Fossati and Prencipe.[13] Blank was prepared by putting 1.0 mL of reagent in test tube and adding 10 µL of distilled water. Standard was prepared by putting 1.0 ml of triglyceride reagent in test tube and adding 10 µL of standard. The reagent was then added to three labeled test tubes and 10 µL of the sample was added. Test tubes were incubated for 10 minutes at 37°C, and absorbance measured at 500 nm.

$$\text{Triglyceride conc. (mg/dL)} = \frac{Absorbance\ of\ test\ sample}{Absorbance\ of\ standard} \times \text{Concentration of std (mg/dl)}$$

LDL: LDL-Cholesterol concentration was determined according to formula by Friendewald *et al*.[14]

$$\text{LDL-Cholesterol} = \text{Total cholesterol} - (\text{HDL-cholesterol} + \text{Triglyceride}/5)$$

Statistical analysis
The results obtained were expressed as Mean ± SD for n = 5 rats/group. The data were analyzed using one-way analysis of variance (ANOVA) and Tukey HSD post-hoc analysis. Results were considered statistically significant when $p < 0.05$.

Results and Discussion

Antidiabetic activity of methanol leaf extracts of Dryopteris filix mas
Effect of *Dryopteris filix* extract on levels of glucose is shown in Table 1. The results obtained show that treatment with alloxan caused an initial significant ($p < 0.05$) increase in blood glucose levels in experimental rats in comparison to the normal rats. The observed significant increase in blood glucose levels was consistent after the 7th, 14th, and 21st day in untreated rats. However, administration of varying doses of *Dryopteris filix-mas* methanol leaf extract in diabetic rats, led to observed significant decreases in blood glucose levels on the 7th, 14th and 21st day in comparison to untreated diabetic rats. The observed ability of this extract to lower the blood glucose levels in diabetic rats compared favorably to that observed in the group treated with metformin. The noticed antidiabetic activity of *Dryopteris filix-mas* could be as a result of the presence of previously discovered phytochemicals[7], such as flavonoids, steroids, tannins, in the plant which are known to possess antidiabetic activity.

Antidyslipidemic activity of methanol leaf extracts of Dryopteris filix mas
The effect of methanol leaf extracts of *Dryopteris filix-mas* on lipid profile of alloxan-induced diabetic rats (Table 2) shows that the plant extract ameliorated the diabetes-induced dyslipidemia observed in the experimental animals. There were significant increases in cholesterol levels in untreated diabetic rats in comparison to the normal rats. Administration of *Dryopteris filix-mas* extract led to decrease in serum cholesterol levels with the groups administered 250 mg/kg and 500mg/kg producing significant decrease ($p<0.05$) in cholesterol levels. The groups administered metformin and 100 mg/kg of *Dryopteris filix-mas* extract also recorded decrease in cholesterol concentration, although the decrease was not statistically significant ($p\geq0.05$). There was also significant increase in triglyceride level in untreated diabetic rats, in comparison to normal rats. Administration of *Dryopteris filix-mas* extract led to significant decrease ($p<0.05$) in serum triglyceride levels in groups administered varying doses of the extracts (100 mg/kg, 250 mg/kg and 500 mg/kg). The decrease was comparable to that observed in group administered metformin.

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Table 1: Blood glucose levels in rats before and after treatment with methanol leaf extracts of *Dryopteris filix-mas*

Groups	Glucose levels (mg/dL)			
	Initial	Day 7	Day 14	Day 21
1	79.25 ± 12.42[a]	79.25 ± 12.42[a]	82.00 ± 12.44[a]	83.50 ± 6.14[a]
2	273.75 ± 18.30[b]	275.65 ± 15.30[b]	279.75 ± 20.3[b]	284.50 ± 10.44[b]
3	273.50 ± 9.51[b]	231.50 ± 22.13[c]	142.75 ± 11.89[c]	98.25 ± 12.52[c]
4	275.50 ± 9.77[b]	242.25 ± 19.43[c]	155.75 ± 12.49[c]	108.00 ± 8.67[c]
5	277.00 ± 6.50[b]	220.50 ± 5.38[c]	138.75 ± 18.87[c]	94.00 ± 10.98[c]
6	259.75 ± 6.39[b]	219.75 ± 6.39[c]	133.75 ± 19.98[c]	90.25 ± 5.05[c]

Values are expressed as Mean ± SD for n = 5 rats per group. Values bearing different superscript on a column differ significantly ($p<0.05$) in comparison to the normal control group when analyzed using one-way analysis of variance.
Group 1: Normal control group.
Group 2: Untreated Diabetic rats (negative control)
Group 3: Diabetic rats administered 500mg/kg of metformin (standard antidiabetic drug).
Group 4: Diabetic rats administered 100mg/kg of *Dryopteris filix* methanol leaf extract.
Group 5: Diabetic rats administered 250mg/kg of *Dryopteris filix* methanol leaf extract.
Group 6: Diabetic rats administered 500mg/kg of *Dryopteris filix* methanol leaf extract.

Table 2: Effect of methanol leaf extracts of *Dryopteris filix-mas* on lipid profile of alloxan induced diabetic rats after 21 days treatment

Groups	CHOL (mg/dL)	TRIG (mg/dL)	HDL (mg/dL)	LDL (mg/dL)
1	216.06 ± 5.79[b]	228.76 ± 21.57[b]	64.75 ± 5.27[b]	105.49 ± 9.83[b]
2	306.15 ± 20.99[a]	321.77 ± 27.02[a]	39.83 ± 8.16[a]	201.97 ± 6.70[a]
3	269.78 ± 18.16[a]	250.35 ± 13.81[b]	51.20 ± 2.98[b]	168.51 ± 8.51[a]
4	267.51 ± 19.90[a]	263.87 ± 19.56[b]	52.12 ± 6.42[b]	162.10 ± 9.75[a]
5	236.27 ± 21.92[b]	257.88 ± 18.30[b]	55.13 ± 7.09[b]	129.56 ± 3.21[b]
6	238.46 ± 43.21[b]	222.60 ± 16.42[b]	51.72 ± 9.65[b]	142.25 ± 3.92[b]

Values are expressed as Mean ± SD for n=5 rats per group. Values bearing different superscript on a column differ significantly ($p<0.05$) when analyzed using one-way analysis of variance (ANOVA)
Group 1: Normal control group.
Group 2: Untreated Diabetic rats (negative control)
Group 3: Diabetic rats administered 500mg/kg of metformin (standard antidiabetic drug).
Group 4: Diabetic rats administered 100mg/kg of *Dryopteris filix* methanol leaf extract.
Group 5: Diabetic rats administered 250mg/kg of *Dryopteris filix* methanol leaf extract.
Group 6: Diabetic rats administered 500mg/kg of *Dryopteris filix* methanol leaf extract.

There was significant increase ($p<0.05$) in LDL level in untreated diabetic rats, in comparison to normal rats. Administration of *Dryopteris filix-mas* extract led to decrease in serum LDL levels with the groups administered 250 mg/kg and 500mg/kg producing significant decrease ($p<0.05$) in LDL levels. The groups administered metformin and 100 mg/kg of *Dryopteris filix-mas* extract also recorded decrease in LDL concentration, although the decrease was not statistically significant ($p\geq0.05$). HDL levels significantly reduced ($p<0.05$) in untreated diabetic rats in comparison to normal rats but administration of varying doses of *Dryopteris filix-mas* extracts significantly ($p<0.05$) increased HDL levels.

The results revealed that methanol leaf extracts of *Dryopteris filix-mas* exhibited significant antihyperglycemic and antidyslipidemic activities in alloxan-induced diabetic rats. The extract's antidiabetic activity was comparable with metformin. Alloxan induces diabetes through two different mechanisms; by selectively inhibiting glucose-induced insulin secretion through the inhibition of the enzyme, glucokinase which is the glucose sensor of the beta cell, and also by inducing the formation of reactive oxygen species (ROS), resulting in selective necrosis of the beta cells.[15,16] Metformin is known to exert it's antihyperglycemic effect by blocking gluconeogenesis, increasing glucose uptake by skeletal muscle,[17] and reducing glucose absorption in the intestinal mucosa.[18-20] The possible mechanisms by which *Dryopteris filix-mas* exerts its antidiabetic activity may be by improving glycemic control, inhibiting adipogenesis and by improving antioxidant status in the experimental animals. Diabetic state appears to be linked to increased cholesterol synthesis. Hyperphagia of diabetes has been hypothesized to induce increased activity of the enzyme, HMG-CoA reductase, in the intestine leading to increased cholesterol synthesis, consequently resulting in higher levels in plasma. Dietary cholesterol also adds up to total cholesterol by increased absorption.[21] The hypertriglyceridemia could be due to higher production rates of triglyceride rich VLDL by the liver and to decreased removal of triglyceride by peripheral tissues-primarily adipose tissue and muscle.[22] Deficiency of insulin leads to high production of triglyceride and the subsequent increased packaging in VLDL. Lower HDL-C in diabetes could be due to reduced Lipoprotein Lipase activity.[22] High LDL in diabetes could be due to increased synthesis of VLDL or impaired removal of VLDL remnant.[22] Impaired receptor mediated clearance of LDL has also been postulated.[22] Administration of methanol leaf extracts of *Dryopteris filix-mas* likely reverse these processes, thereby leading to improved lipid profile in diabetic rats.

Conclusion

The findings from this study revealed that *Dryopteris filix-mas* methanol extract possess antidiabetic and antidyslipidemic activities in alloxan induced diabetic rats. The extract has beneficial effect on level of blood glucose, and ameliorated the diabetes-induced changes in lipid profile.

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Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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References

1. Roper NA, Bilous RW, Kelly WF, Unwin NC, Connolly VM. Excess mortality in a population with diabetes and the impact of material deprivation: longitudinal, population-based study. Br Med J. 2001; 332(7299):1389-1393.
2. NCD Risk Factor Collaboration. Worldwide trends in diabetes since 1980: A pooled analysis of 751 population-based studies with 4.4 million participants. Lancet. 2016; 387(10027):1513-1530.
3. Schofield JD, Liu Y, Rao-Balakrishna P, Malik RA, Soran H. Diabetes Dyslipidemia. Diabetes Ther. 2016; 7(2):203-219.
4. Bahiru E, Hsiao R, Phillipson D, Watson KE. Mechanisms and treatment of dyslipidemia in diabetes. Curr Cardiol Rep. 2021; 23(4):26.
5. Sofowora A. Medicinal plants and traditional medicine in Africa. John Wiley & Sons ltd. New York. 1982.
6. Tagarelli G, Tagarelli A, Piro A. Folk medicine used to heal malaria in Calabria (southern Italy). J Ethnobiol Ethnomed. 2010; 6(27):1-16.
7. Uwumarongie HO, Enike MA, Bafor EE. Pharmacognostic evaluation and gastrointestinal activity of *D. filix-mas*. Ewemen J Herb Chem Pharmacol Res. 2016; 2(1):19-25.
8. Erhirhie EO, Ilodigwe EE, Ajaghaku DL, Umeokoli BO, Eze PM. Antioxidant Activities of the Leaf Extract and Fractions of Dryopteris filix-mas (L.) Schott could be Attributed to The Abundance of Polyphenol Compounds. Biol Med Nat Prod Chem. 2020; 9(1):1-6.
9. Erhirhie EO, Emeghebo CN, Ilodigwe EE, Ajaghaku DL, Umeokoli BO, Eze PM, Ngwoke KG, Okoye FBC. *Dryopteris filix-mas* (L.) Schott ethanolic leaf extract and fractions exhibited profound anti-inflammatory activity. Avicenna J Phytomed. 2019; 9(4):396-409.
10. Erhirhie E, Anyim C, Okezie M, Igboeme S, Ilodigwe E. Evaluation of Wound Healing Properties of Dryopteris filix-mas Leaf and Root Extracts on Albino Rats. *Iran J Pharm Sci*. 2021; 17(1):79-90.
11. Allain CC, Poon LS, Chan CS, Richmond W, Fu PC. Enzymatic determination of total serum cholesterol. Clin Chem. 1974; 20(4):470-475.
12. Castelli WP, Doyle JT, Gordon T, Hames CG, Hjortland MC, Hulley SB, Kagan A, Zukel WJ. HDL Cholesterol and Other Lipids in Coronary Heart Disease: Cooperative Lipoprotein Phenotyping Study. Circul. 1977; 55(5):767-772.
13. Fossati P and Prencipe L. Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. Clin Chem. 1982; 28(1):2077-2080.
14. Friendewald WT, Levy RT, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma without use of the preparative ultracentrifuge. Clin Chem. 1972; 18(6):499-502.
15. Lenzen S. The mechanisms of alloxan- and streptozotocin-induced diabetes. Diabetologia. 2008; 51(2):216-226.
16. Macdonald IO, Mohammed AA, Adeboye AO. Alloxan-induced diabetes, a common model for evaluating the glycemic-control potential of therapeutic compounds and plants extracts in experimental studies. *Med.* 2017; 53(6):365-374.
17. Musi N, Hirshman MF, Nygren J. Metformin increases AMP-activated protein kinase activity in skeletal muscle of subjects with type 2 diabetes. Diabetes 2002; 51(7):2074-2081.
18. Ikeda T, Iwata K, Murakami H. Inhibitory effect of metformin on intestinal glucose absorption in the perfused rat intestine. Biochem Pharmacol. 2000; 59(7):887-890.
19. Song, R. Mechanism of Metformin: A tale of two sites. Diabetes Care. 2016; 39(2):187-189.
20. Buse JB, DeFronzo RA, Rosenstock J. The primary glucose-lowering effect of metformin resides in the gut, not the circulation: results from short-term pharmacokinetic and 12-week dose-ranging studies. Diabetes Care. 2016; 39(2):198–205.
21. Saudek CD and Young NL. Cholesterol metabolism in diabetes mellitus. Diabetes. 1981; 30(2):76-81.
22. Nikkila EA and Hormila P. Serum lipids and lipoproteins in insulin treated diabetes. Diabetes. 1978; 27(11):1078-1086.