

Antidiabetic Activity of *Diospyros mespiliformis* on Alloxan-Induced Diabetic Rats

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Abstract

The rate of morbidity and mortality attributed to diabetes has become a concern and challenge for individuals and governments. The availability, affordability, and efficacy of plant-based drugs make them an attractive choice for diabetic management in low-income countries and rural communities. Thus, their application in folkloric medicine for diabetic management. This study investigated the antidiabetic activity of the crude ethanol extract (CRE), ethyl acetate (EAF), and aqueous (AQF) fractions *Diospyros mespiliformis* (DM) in alloxan-induced diabetic rats to justify its acclaimed applications in folkloric medicine. The effect of the plant extract and its fractions on the aspartate aminotransferase, glutamyl aminotransferase, albumin, urea, creatinine, electrolytes, and lipid profile was determined by biochemical assay methods. The result showed a significant ($p < 0.05$) decrease in fasting blood glucose for all the extracts, while the aspartate aminotransferase, gamma-glutamyl transferase, and albumin were significantly ($p < 0.05$) decreased in the EAF only. The urea and creatinine levels of the CRE and AQF were decreased significantly ($p < 0.05$), while K^+ , Cl^- , and HCO_3^- levels decreased significantly ($p < 0.05$) for the treatment groups. Furthermore, a significant ($p < 0.05$) decrease in total cholesterol and triglyceride was observed for the EAF. Conclusively, DM exhibited significant hypoglycemic and hypolipidemic potential with improved lipid profile and hepato-renal function. Thus, the observed antidiabetic activity of the plant might justify its acclaimed utilization in the treatment/management of diabetes and its related ailment.

Keywords: Alloxan, Antidiabetic activity, Antihyperglycemic, Antihyperlipidemic, *Diospyros mespiliformis*, Electrolytes

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INTRODUCTION

Diabetes is a metabolic ailment composed of a collection of metabolic disorders associated with persistent hyperglycemia accompanied by acute and chronic complications, including nephropathy, retinopathy, and neuropathy.¹ It has been regarded to be among the leading causes of morbidity and mortality, yet treatment

remains a challenge.² The recommended treatment option is proper diet and exercise, though drugs are used in necessary situations.³ Worldwide, the ailment continues to be a concern to individuals and governments, creating financial and welfare burdens for the concerned entity.² A 2021 global report showed diabetic prevalence to be up to

10% in adults between the ages of 20 and 72 years, and the expected projection to be 12% by 2045, irrespective of gender.² Moreover, the global expenditure on diabetes and related ailments was estimated to rise to 1,054 billion USD by 2045 from the reported 966 billion USD in 2021.² Thus, the cost and affordability of antidiabetic drugs are also a consideration, especially for low-income countries and rural communities.

Diabetes as a multifactorial metabolic disorder creates more challenges in treatment, often leading to combined therapy and multiple doses of antidiabetic agents.⁴ The continued use of antidiabetic drugs is often rendered undesirable to individuals, leading to prospects for other treatment options. Moreover, these drugs were reported to possess undesirable adverse effects, including hypoglycemia, bloating, nausea, and anorexia.⁴ Furthermore, for rural communities, in addition to poverty, access to proper healthcare is a challenge, often relying on herbal formulations for therapeutic purposes.⁵ Thus, they often indulge in the use of plant-based drugs as an alternative to achieve therapeutic goals. Medicinal plants in different preparations and formulations of varying efficacy are used in folkloric medicine for the treatment of ailments, including diabetes.⁵ The pharmacological activities of these plants are attributed to their secondary metabolite contents, including flavonoids and saponins.⁶ Moreover, the diverse and synergistic effects of plant-based drugs make them an attractive choice of therapy, considering diabetes as a collection of multiple metabolic disorders.⁷ Previous reports showed the use of plants in the management of diabetes, including *Diospyros mespiliformis*.⁸⁻¹⁰

Diospyros mespiliformis is a tree of African origin, often referred to as African ebony, which harbors fruits consumed as food.¹¹ In folkloric medicine, the plant parts are employed in the management of diabetes¹² and its related conditions, including inflammation¹³ and oxidative stress.¹¹ Thus, in our study, we justified the antidiabetic application of the plant in folkloric medicine by investigating the antidiabetic activity of its crude ethanol extract, ethyl acetate, and aqueous fractions in alloxan-induced diabetic rats considering the effects in the liver, kidney, and lipid profile of the diabetic rats.

METHOD

Plant sample and preparation

The plants' stem bark sample was obtained from the Mayo-belwa area of Adamawa State, Nigeria, and authenticated in the Forest Technology Department of Adamawa State Polytechnic, where a voucher specimen (no. ASP/FT/091) was deposited. The stem bark was air-dried and powdered with a blender.

Experimental animals

Male albino rats were obtained from Hema Farms Nigeria Ltd., Yola, Adamawa State. The rats weighing 150 ±20 g were kept under normal dark/light cycles and acclimatized for 7 days with free access feed (Finisher pellet, Chikun Feed, Crown Flour Mill Ltd, Lagos) and water. The Norwegian National Committee for Research Ethics in Science and Technology (NENT) 2018 ethical guideline was strictly followed for all the experimental procedures.¹⁴

Extraction and fractionation

Exactly 1 kg of the sample was macerated for 7 days in 70% ethanol (v/v) and filtered, followed by drying under reduced pressure at 40 °C with a rotary evaporator (Buchi Rotavapor R-200) yielding 95 g of the crude ethanol extract (CRE). Exactly 50 g of the CRE was suspended to complete

dissolution in 200 ml of distilled water and continuously partitioned in a separating funnel by the addition of ethyl acetate until the formation of a clear ethyl acetate layer yielding the ethyl acetate fraction (EAF). The remaining aqueous layer was regarded as the aqueous fraction (AQF). Both the EEF and AQF were subjected to the same drying conditions as the CRE, yielding 10.60 g and 36.80 g of the EEF and AQF, respectively.

Induction of diabetes

Alloxan monohydrate (Oxford Lab Fine Chem LLP, India) prepared in normal was used to induce diabetes at 150 mg/kg body weight intraperitoneally to overnight fasted diabetic rats. Diabetes was confirmed in rats exhibiting fasting blood glucose (FBG) above 200 mg/dl, and treatment was initiated immediately.¹⁵

Experimental Design

The animals were randomly grouped into 6 groups, and treatment was administered by intragastric tube daily for 21 days. Group I (non-diabetic) and II (diabetic) received 10 ml/kg body weight. Group III (diabetic) received 150 mg/kg metformin while IV (CRE), V (EAF), and VI (AQF) were all diabetic and received 300 mg/kg body weight CRE, EAF, and AQF, respectively. The standard drug metformin [(Diabetmin®) Hovid Pharmaceuticals Ltd, Nigeria] was employed as a positive control. The FBG was read at the beginning and end of the experiment by tail vein puncture using a glucometer (SD CodeFree™, SD Biosensor, Inc., Korea), whereas the body weight was measured weekly for 3 weeks. The rats were pre-anesthetized with chloroform before blood collection, and then they were placed into lithium-heparin tubes by cardiac puncture and centrifuged for 20 minutes at 3000 rpm, separating serum from the cells.

Biochemical assay

The following biochemical parameters were determined by previously described methods as follows:

1. Aspartate aminotransferase (AST) described by Reitmann.¹⁶
2. Gamma-glutamyl transferase (GGT) described by Szasz.¹⁷
3. Albumin concentration described by Grant.¹⁸
4. Urea concentration described by Chaney and Marbach.¹⁹
5. Creatinine concentration described by Bartels.²⁰
6. The estimation of electrolytes was according to the kit's manufacturer's instructions.
7. Total Cholesterol (TC) as described by Stein.²¹
8. Triglyceride Concentration (TG) as described by McGowan.²²
9. High-density Lipoprotein-Cholesterol (HDL-C) as described by Warnick and Albers.²³
10. Low-density Lipoprotein Cholesterol (LDL-C) as described by Friedewald.²⁴

Statistics

The data obtained was statistically evaluated with Statistical Package for Social Sciences (SPSS) software version 22 and expressed as mean \pm standard mean error. One-way analysis of variance was employed to evaluate the difference among the groups and subsequently evaluated by Turkey's multiple comparison tests at $p < 0.05$ significant level.

RESULTS AND DISCUSSION

FBG and body weight

The effect of the CRE, EAF, and AQF on the FBG and body weight of the diabetic rats is presented in Figure 8. The diabetic

groups demonstrated a significant ($p < 0.05$) rise in initial FBG than the naïve control. However, at the end of the experiment, the negative control exhibited significantly ($p < 0.05$) higher FBG than the treatment groups, with no significant ($p > 0.05$) difference among them. The AQF exhibited a significant ($p < 0.05$) increase in body weight than the EAF at weeks 1 and 2, while the naïve control was at week 3. However, the negative control demonstrated a significant ($p < 0.05$) decrease in body weight than all the groups at week 3. The administration of alloxan to the rats led to a rise in FBG that was persistent to the end of the

experiment for the negative control. This was due to the necrotic action of alloxan on the β -cells inhibiting insulin secretion and apoptosis to β -cells via the generation of free radicals.¹⁵ The observed decreased hyperglycemia for the treatment groups might be attributed to the reversal of the alloxan-induced damage to the β -cells via the antioxidant activity of the extracts. The improvement in β -cell function might further be attributed to the improved body weight observed in the treated groups as opposed to the negative control with decreased body weight due to muscle wasting.

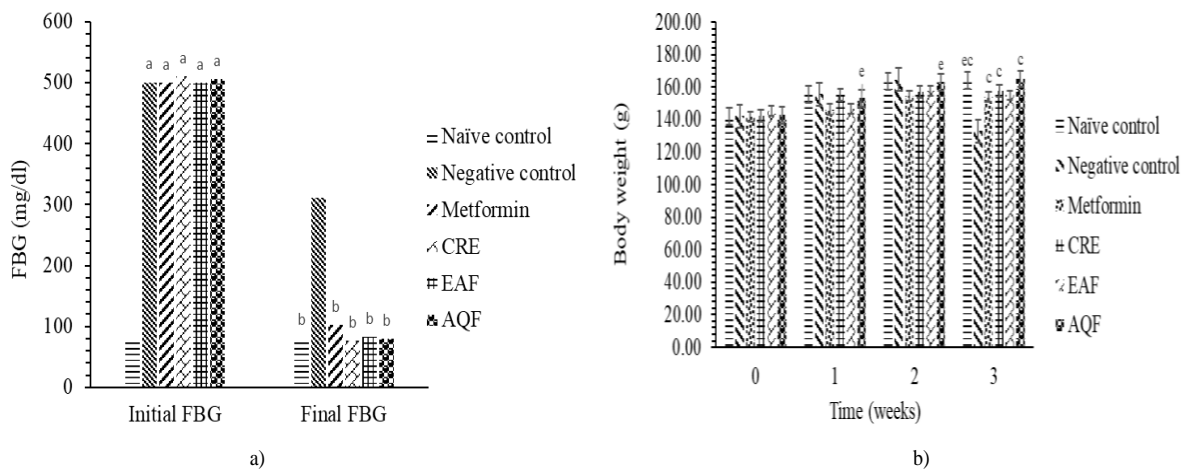


Figure 1. Effects of DM on the; a) FBG and b) Body weights. Values with a and c superscripts are significantly ($p < 0.05$) higher than naïve and negative control respectively while values with b superscripts are significantly ($p < 0.05$) lower than negative control. Values with e superscripts are significantly ($p < 0.05$) higher than EAF

AST, GGT, and albumin

The effect of the CRE, EAF, and AQF on the levels of serum AST, GGT, and albumin is presented in Table 4. A significant ($p < 0.05$) rise in AST level was observed for the negative control (150.00 ± 2.88 IU/L) compared to the naïve control (116.40 ± 8.64 IU/L), CRE (102.80 ± 0.97 IU/L), and EAF (82.20 ± 4.92 IU/L). Furthermore, a significant ($p < 0.05$) decrease in the AST

level was observed for the CRE and EAF compared to the AQF (144.00 ± 1.05 IU/L) and Metformin (135.60 ± 5.96 IU/L), with the latter significantly ($p < 0.05$) decreased than the naïve control. The GGT level of the negative control (12.00 ± 0.28 IU/L) was significantly ($p < 0.05$) increased compared to the other groups.

Table 1. Effects of CRE, EAF, and AQF of DM on the serum levels of AST, GGT, and albumin

Groups	AST (IU/L)	GGT (IU/L)	Albumin (g/L)
Naïve control	116.40 ± 8.64	8.32 ± 0.42 ^b	25.40 ± 0.93 ^b
Negative control	150.00 ± 2.88 ^a	12.00 ± 0.28	31.00 ± 1.05
Metformin	135.60 ± 5.96 ^a	9.08 ± 0.40 ^b	26.40 ± 0.98 ^b
CRE	102.80 ± 0.97 ^{bcd}	7.80 ± 0.70 ^b	33.20 ± 0.37 ^{ad}
EAF	82.20 ± 4.92 ^{abc}	7.00 ± 0.55 ^{bc}	25.00 ± 1.30 ^b
AQF	144.00 ± 1.05 ^{ade}	6.6 ± 0.68 ^{bc}	28.20 ± 0.37 ^d

Values are expressed as mean ± SEM: n = 5

Values in the same column with b and c superscripts were significantly ($p < 0.05$) lower than the negative control and Metformin group, respectively, while those with a, d, and e were higher than naïve control, EAF, and CRE groups, respectively.

Moreover, the GGT levels of EAF (7.00 ± 0.55 IU/L) and AQF (6.6 ± 0.68 IU/L) were significantly ($p < 0.05$) decreased compared to the Metformin (9.08 ± 0.40 IU/L). Additionally, the albumin level of the negative control (31.00 ± 1.05 IU/L) was significantly ($p < 0.05$) increased than the naïve control (25.40 ± 0.93 IU/L), Metformin (26.40 ± 0.98 IU/L), and EAF (25.00 ± 1.30 IU/L). The albumin level of the EAF was significantly ($p < 0.05$) decreased compared to CRE (33.20 ± 0.37 IU/L) and AQF (28.20 ± 0.37 IU/L). The observed increased levels of AST, GGT, and albumin are indicators of liver injury or dysfunction²⁶ observed in the negative control, which might be attributed to hyperglycemia.²⁷ Thus, the decreased level observed for the treatment groups might be due to improved β -cell function and glycemic control.

Urea, creatinine, electrolytes

Table 5 reveals the effect of the CRE, EAF, and AQF on the serum urea and creatinine levels. A significantly ($p < 0.05$) higher urea level (14.66 ± 1.29 mM/L) was demonstrated by the EAF compared to the other groups. The urea level of the naïve control (6.94 ± 0.09 mM/L) and CRE (6.72 ± 0.45 mM/L) was significantly ($p < 0.05$) decreased compared to the negative control (9.70 ± 0.19 mM/L). The naïve control (50.00 ± 2.39 μ M/L), Metformin (58.60 ± 3.42 μ M/L), and AQF (71.40 ± 1.17 μ M/L) exhibited a significant ($p < 0.05$)

decrease in creatinine levels than the negative control (94.60 ± 10.22 μ M/L). Additionally, the creatinine level of the EAF (99.60 ± 2.36 μ M/L) exhibited a significant ($p < 0.05$) increase compared to Metformin. The Na⁺ level of all the groups wasn't significantly ($p > 0.05$) different. However, a significant ($p < 0.05$) increase in K⁺ level was exhibited by the treatment groups compared to the naïve (9.54 ± 0.58 mEq/L) and negative (8.68 ± 0.27 mEq/L) control. Furthermore, all the diabetic groups showed a significant ($p < 0.05$) decrease in Cl⁻ levels compared to the naïve control (102.8 ± 1.83 mM/L). The negative control (17.20 ± 0.49 mM/L) showed a significant ($p < 0.05$) rise in HCO₃⁻ level than the other groups, with all the treatments showing a significant ($p < 0.05$) decrease compared to the naïve control (28.60 ± 0.25 mM/L).

Kidney dysfunction can be marked by increased urea and creatinine levels and metabolic waste filtered from blood by the kidney.²⁸ In our study, a significant decrease in the urea level might indicate an improved renal function for the CRE and Metformin groups with a decreased creatinine level for the AQF. In diabetes, increased plasma osmolarity and impaired renal function associated with hyperglycemia create electrolyte imbalance.²⁹ All the treatment groups presented hypokalemia, which might be induced by the treatments.³⁰ Furthermore, the diabetic groups

exhibited hypochloremia, which might be due to ketoacidosis-induced alkalosis.³¹ Moreover, all the treatment groups

presented improved decreased bicarbonate due to ketoacidosis³¹ observed in the negative control group.

Table 2. Effects of CRE, EAF, and AQF of DM on the serum levels of urea, creatinine, and electrolytes

Groups	Urea (mM/L)	Creatinine (µM/L)	Na ⁺ (mEq/L)	K ⁺ (mEq/L)	Cl ⁻ (mM/L)	HCO ₃ ⁻ (mM/L)
Naïve control	6.94 ± 0.09	50.00 ± 2.39	141.40 ± 1.08	9.54 ± 0.58	102.8 ± 1.83	28.60 ± 0.25
Negative control	9.70 ± 0.19 ^a	94.60 ± 10.22 ^a	142.00 ± 0.55	8.68 ± 0.27	96.80 ± 0.80 ^e	17.20 ± 0.49 ^e
Metformin	7.82 ± 0.44	58.60 ± 3.42 ^b	141.60 ± 0.40	6.44 ± 0.14 ^{ef}	98.00 ± 0.45 ^e	25.20 ± 1.69 ^f
CRE	6.72 ± 0.45 ^{bd}	91.2 ± 4.13 ^{ac}	141.20 ± 1.66	6.04 ± 0.33 ^{ef}	95.60 ± 1.21 ^e	21.80 ± 0.37 ^{ef}
EAF	14.66 ± 1.29 ^{abc}	99.60 ± 2.36 ^{ac}	140.40 ± 0.93	7.24 ± 0.13 ^{ef}	97.00 ± 0.89 ^e	22.20 ± 0.20 ^{ef}
AQF	8.10 ± 0.07 ^d	71.40 ± 1.17 ^{bd}	141.00 ± 0.71	7.06 ± 0.19 ^{ef}	97.40 ± 0.40 ^e	24.40 ± 0.75 ^{ef}

Values are expressed as mean ± SEM: n = 5

Values in the same column with a, c, and f superscripts were significantly (p < 0.05) higher than naïve control, Metformin, and negative control groups, respectively, while those with b, d, and e superscripts were significantly (p < 0.05) lower than negative control, EAF, and naïve control groups respectively.

TC, TG, HDL-C, and LDL-C

Table 6 reveals the effects of the CRE, EAF, and AQF on the serum TC, TG, HDL-C, and LDL-C levels. A significantly (p < 0.05) higher TC level was exhibited by the negative control (85.02 ± 3.78 mg/dl) than the other groups without a significant (p > 0.05) difference among the treatment groups. Moreover, the TG level of the negative control (275.90 ± 6.29 mg/dl) was significantly (p < 0.05) increased compared to the other groups except for the CRE (201.14 ± 31.14 mg/dl) with a significant (p < 0.05) rise than EAF (110.36 ± 7.23 mg/dl) and AQF (90.78 ± 17.21 mg/dl). The CRE and Metformin (28.08 ± 4.34 mg/dl) exhibited a significantly (p < 0.05) decreased HDL-C level than the naïve (101.4 ± 7.99 mg/dl) and negative (99.06 ± 7.04 mg/dl) control while the EAF (94.38 ± 6.09 mg/dl) and AQF (82.68 ± 2.887 mg/dl)

were higher than Metformin only. The LDL-C level of Metformin (28.08 ± 4.34 mg/dl) was significantly (p < 0.05) decreased compared to the naïve control (64.74 ± 13.93 mg/dl) and EAF (63.96 ± 10.58 mg/dl).

The loss of glycemic control in diabetes can lead to dyslipidemia with a rise in TC, TG, LDL-C, and decreased HDL-C levels. However, the LDL-C might remain unchanged.³² An increased TC and TG level might indicate dyslipidemia; however, all the treatment groups demonstrated improved levels, thus improving renal functions.

Table 3. Effects of CRE, EAF, and AQF of DM on the serum levels of TC, TG, HDL-C, and LDL-C

Groups	TC (mg/dl)	TG (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)
Naïve control	52.26 ± 2.65 ^b	131.72 ± 15.26 ^b	101.4 ± 7.99 ^c	64.74 ± 13.93 ^c
Negative control	85.02 ± 3.78	275.90 ± 6.29	99.06 ± 7.04	49.92 ± 2.87
Metformin	52.26 ± 3.40 ^b	131.72 ± 10.30 ^b	41.34 ± 5.02 ^b	28.08 ± 4.34
CRE	60.06 ± 3.65 ^b	201.14 ± 31.14	67.08 ± 5.43 ^{bf}	49.14 ± 4.71
EAF	49.92 ± 4.51 ^b	110.36 ± 7.23 ^{bd}	94.38 ± 6.09 ^{ce}	63.96 ± 10.58 ^c
AQF	47.58 ± 0.78 ^b	90.78 ± 17.21 ^{bd}	82.68 ± 2.887 ^c	53.04 ± 6.36

Values are expressed as mean ± SEM: n = 5

Values in the same column with a, c, and e superscripts were significantly ($p < 0.05$) higher than naïve control, Metformin, and CRE groups, respectively, while those with b, d, and f were significantly ($p < 0.05$) lower than negative control, CRE, and naïve control groups respectively.

CONCLUSION

DM exhibited significant hypoglycemic and hypolipidemic potential with improved lipid profile and hepato-renal function. Thus, the observed antidiabetic activity of the plant might justify its acclaimed utilization in the treatment/management of diabetes and its related ailment.

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CONFLICT OF INTEREST

All authors declare that there is no potential conflict of interest with the research, authorship, and/or article publication.

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