



Crude Ethanol Extract of *Diospyros mespiliformis* Hochst. ex A. DC. Ebenaceae Leaf and Its Fractions Ameliorate Hyperglycemia and Hyperlipidemia in Alloxan-induced Diabetic Rats

Mubarak Muhammad Dahiru^{1,*}, Neksumi Musa²

¹Adamawa State Polytechnic, School Science and Technology, Department of Pharmaceutical Technology, 640101, Yola, Nigeria

²Adamawa State Polytechnic, School Science and Technology, Department of Science Laboratory Technology, 640101, Yola, Nigeria

*Corresponding author: mubaraq93@adamawapoly.edu.ng

Abstract

This study investigated the antidiabetic activity of the crude ethanol extract of *Diospyros mespiliformis* (DM) and its ethyl acetate (EEF) and aqueous (AQF) fractions on alloxan-induced diabetic rats. The result indicated a significant ($p < 0.05$) decrease and improvement in fasting blood glucose (FBG) level and body weight, respectively. The treatment groups exhibited a significantly ($p < 0.05$) decreased aspartate aminotransferase (AST) level (100-117 IU/L) and decreased gamma-glutamyl transferase (GGT) level in the EEF (4.80 ± 1.02 IU/L) and AQF (5.80 ± 0.80 IU/L) groups only. All the extract-treated groups exhibited a significant increase ($p < 0.05$) in urea and creatinine levels than the naïve control group (6.94 ± 0.20 mM/L). Moreover, the Na^+ remained significantly ($p > 0.05$) unchanged while the K^+ level was significantly ($p < 0.05$) increased for the treatment groups. The HCO_3^- of the treated groups increased significantly ($p < 0.05$) except for the AQF (21.60 ± 1.63 mM/L) group. The total cholesterol and triglyceride levels decreased significantly ($p < 0.05$) for the treatment groups while the high-density lipoprotein-cholesterol (HDL-C) and low-density lipoprotein-cholesterol (LDL-C) levels remained unchanged but significantly higher than the metformin group. DM possesses significant hypoglycemic and hypolipidemic activity lowering hyperglycemia and hyperlipidemia, and improving body weight and diabetic markers.

Keywords: Alloxan, Antidiabetic activity, *Diospyros mespiliformis*, Hypoglycemia, Hypolipidemia

Received: 03 February 2024

Accepted: 01 December 2024

DOI: <https://doi.org/10.25026/jtpc.v8i2.625>



Copyright (c) 2024, Journal of Tropical Pharmacy and Chemistry. Published by Faculty of Pharmacy, University of Mulawarman, Samarinda, Indonesia. This is an Open Access article under the CC-BY-NC License.

How to Cite:

Dahiru, M. M., Musa, N., 2024. Crude Ethanol Extract of *Diospyros mespiliformis* Hochst. ex A. DC. Ebenaceae Leaf and Its Fractions Ameliorate Hyperglycemia and Hyperlipidemia in Alloxan-induced Diabetic Rats. *J. Trop. Pharm. Chem.* **8**(2). 136-145. DOI: <https://doi.org/10.25026/jtpc.v8i2.625>

1 Introduction

Diabetes is a state of altered metabolism and is regarded as a leading cause of mortality and morbidity especially in developing and low-income countries [1]. Among others, persistent hyperglycemia has been identified as one of the culprits that alter the normal metabolic state and subsequently result in a diabetic state [1]. A diabetic state results in the alteration of not only carbohydrate metabolism but also lipid metabolism, thus, further complicating its management strategies [2]. Notable complications of the disease include acute and chronic complications, however, the latter which include cardiovascular complications, neuropathy, and nephropathy pose a major challenge in the treatment [1]. The normal recommendation is often improvement in diets and exercise; however, therapeutics are regarded as an additional option to achieve glycemic control [3]. The use of therapeutic is often complicated by the alteration of the metabolic state which leads to further challenges, including the choice of drugs, multiple doses, and combined therapies [4]. In diabetic management, the use of antidiabetic drugs might further complicate treatment as they are often associated with undesirable effects. Thus, continuous use might not be considered [4]. Moreover, for low-income countries, challenges including the cost of the drugs lead to prospects for affordable alternatives such as plant-based drugs [5].

The affordability and availability in addition to minimized side effects of medicinal plants make them alternatives to modern drugs in diabetic management [5]. Medicinal plants of different efficacies were reported as alternatives in folkloric diabetic management

prepared in various forms including decoction, maceration, and infusions [6]. Furthermore, previous literature reported the application of plants in the management of diabetes-related or induced ailments in addition to diabetic management [7-13]. The capability of these plants to relieve and minimize symptoms of diabetes is attributed to their phytochemical components which are secondary metabolites produced by plants for purposes, including protection against weather conditions and predators [14]. Secondary metabolites of various types, including saponins and flavonoids are abundant in plants and were previously reported to exert different individual and synergistic pharmacological effects [15-17]. These metabolites target different proteins associated with the pathology of diabetes [18-21]. Plant-based drugs might relieve diabetic symptoms through interaction with several pathways and different molecules [18, 22]. Thus, an approach to relieve diabetic symptoms by medicinal plants might be viewed as a better alternative as they can target the different alterations caused by diabetes while at the same time bringing about collective relief and minimizing chronic complications. Previous studies revealed the anti-diabetic effects of some plants in diabetic management including hypoglycemic [21], hypolipidemic [11], anti-inflammatory [23], and antioxidant effects [10, 23, 24].

D. mespiliformis (DM) is native to Africa and associated with different pharmacological applications in folkloric healing including antidiabetic purposes [25]. It grows up to 25 meters with long branches and harbors fruits often used as food. Different classes of secondary metabolites were reported from the plant, including flavonoids and saponins,

previously reported to exert antidiabetic effects [8]. In this study, we investigated the antidiabetic activity of the crude ethanol extract, ethyl acetate, and aqueous fractions of *D. mespiliformis* in alloxan-induced diabetic rats.

2 Methods

2.1 Materials

DM was identified by a Forest Technologist from Adamawa State Polytechnic after collection from the Mayo-belwa Area of Adamawa State. A voucher specimen (ASP/FT/23/009) was deposited in the Forestry Technology Department.

2.2 Drugs, reagents, and chemicals

Alloxan (monohydrate): Oxford Lab Fine Chem LLP (India) was used for the diabetic induction. Metformin: Diabetmin® (Hovid Pharmaceuticals Ltd, Nigeria) was used as a standard antidiabetic drug. AST, GGT, albumin, cholesterol, triglycerides, HDL-C, urea, creatinine, and electrolytes kits (Randox Laboratories Ltd, Antrim, UK). All other chemicals and reagents used were of AnalaR.

2.3 Experimental animals

Male Wistar rats weighing 140 ± 30 were obtained from Hema Farms Nigeria Ltd, Yola, Adamawa State. The animals were maintained under standard conditions of dark/light circles with free access to a standard diet (Finisher pellet, Chikun Feed, Crown Flour Mill Ltd, Lagos) and water. All animal experimental procedures were conducted according to the ethical guidelines of the Norwegian National Committee for Research Ethics in Science and Technology (NENT) 2018 [26].

2.4 Extract preparation

The extracts and fractions were prepared as we described previously [19]. Briefly, one kilogram of the sample was subjected to one-week maceration 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). The fractions were obtained by suspending 50 g of the CRE to

complete dissolution in 200 ml of distilled water, followed by continuous partitioning in a separating funnel *via* ethyl acetate addition until a clear ethyl acetate layer was formed, 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 condition as the CRE to yield 10.60 g and 36.80 g of the EEF and AQF, respectively.

2.5 Induction of diabetes

Alloxan monohydrate dissolved in normal saline was intraperitoneally injected into overnight fasted rats at 150 mg/kg body weight to induce diabetes. Diabetes was confirmed in rats with FBG > 200 mg/mL [27]. Treatment was initiated after confirmation of diabetes.

2.6 Experimental design

Table 1 reveals the groups and three weeks of daily treatments administered to the rats *via* intragastric tube. The FBG was recorded initially and on the last day of treatment from the tail vein *via* puncture with a few drops added to the glucometer strip and glucose concentration was read from the glucometer (SD CodeFree™, SD Biosensor, Inc., Cheongju, Korea). The body weight was recorded weekly for three weeks. The blood sample was taken from anesthetized rats *via* cardiac puncture into lithium-heparin tubes and subjected to 20 minutes of centrifugation at 3000 rpm to separate the serum from cells for analysis.

Table 1 Groups and Treatments Administered

Groups	Description	Treatment received
1	Naïve control	10 mL distilled water
2	Negative control (diabetic)	10 mL distilled water
3	Standard control (diabetic)	150 mg/kg b. wt. Metformin
4	Treatment I (diabetic)	300 mg/kg b. wt. CR.
5	Treatment II (diabetic)	300 mg/kg b. wt. EEF
6	Treatment III (diabetic)	300 mg/kg b. wt. AQF

2.7 Biochemical assay analysis

The protocol of Reitmann [28] and Szasz [29] were employed for AST and GGT levels, respectively while the estimation of albumin was as previously described by Grant [30].

Quantitation of urea and creatinine was done as described by Chaney and Marbach [31] and Bartels et al. [32], respectively. TC and TG were *via* the procedures described by Stein [33] and McGowan et al. [34], respectively while the high-density lipoprotein-cholesterol (HDL-C) was quantified by the procedure described previously [35]. Lastly, the low-density lipoprotein cholesterol (LDL-C) was quantified by the protocol described by Friedewald et al. [36] while the serum electrolyte levels were as described in the kit's manufacturer's instructions.

2.8 Statistics

The results obtained were expressed as mean \pm standard error of the mean (\pm SEM) and statistically evaluated using the Statistical Package for Social Sciences (SPSS) version 22 software by one-way analysis of variance

followed by Tukey's multiple comparison tests at $p < 0.05$ level of significance.

3 Results and Discussion

Figure 1a presents the effects of the CR, EEF, and AQF on the FBG. A significantly ($p < 0.05$) higher initial FBG was exhibited by the diabetic groups than the naïve control group (82.44 mg/dl) without any significant difference ($P > 0.05$) between the final FBG of the naïve and treated groups. Notably, the treatment groups exhibited a significantly lower ($p < 0.05$) final FBG than the negative control (265.2 mg/dl) after 21 days. Figure 1b presents the effect of the CR, EEF, and AQF on body weight. The initial body weights of the rats weren't significantly ($p > 0.05$) different among the groups initially up to two weeks of the study. However, a significant ($p < 0.05$) decrease in body weight (133 g) was exhibited by the negative control group after three weeks.

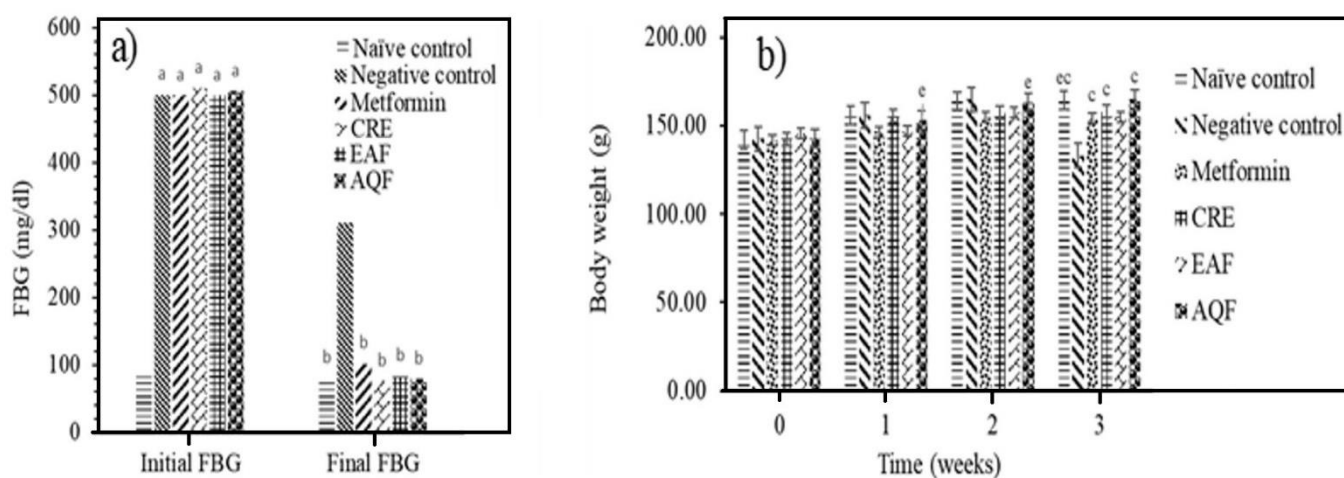


Figure 1 Effect of DM on the; a) FBG and b) Body weights. Values with ^a and ^c superscripts are significantly ($p < 0.05$) higher than the naïve and negative control, respectively while values with ^b superscripts are significantly ($p < 0.05$) lower than the negative control

Table 2 Effect of the CR, EEF, and AQF of DM on the Serum AST, GGT, and Albumin Levels

Groups	AST (IU/L)	GGT (IU/L)	Albumin (g/L)	Urea (mM/L)	Creatinine (μ M/L)
Naïve	118.80 \pm 9.09 ^b	8.24 \pm 0.19 ^b	25.00 \pm 1.58	6.94 \pm 0.20	50.00 \pm 5.34
Negative control	149.40 \pm 3.47	11.40 \pm 0.51	30.20 \pm 0.86 ^e	9.70 \pm 0.42 ^{ef}	94.60 \pm 12.84 ^e
STD (Metformin)	134.00 \pm 7.31	8.60 \pm 0.51	27.20 \pm 0.74	7.82 \pm 0.20	58.60 \pm 7.64 ^e
CR	100.60 \pm 4.12 ^{bc}	8.80 \pm 0.73	31.60 \pm 0.51 ^{ef}	9.96 \pm 0.40 ^{ef}	87.00 \pm 5.34 ^{ef}
EEF	117.60 \pm 5.78 ^b	4.80 \pm 1.02 ^{abcd}	31.40 \pm 0.51 ^{ef}	12.28 \pm 0.92 ^{efgh}	71.80 \pm 4.15 ^{be}
AQF	117.00 \pm 8.72 ^b	5.80 \pm 0.80 ^{bd}	31.20 \pm 0.37 ^{ef}	13.00 \pm 1.51 ^{efgh}	51.40 \pm 8.88 ^{bd}

Values are expressed as mean \pm SEM: n = 5

Diabetes induction *via* alloxan led to a significantly elevated FBG by inhibiting insulin secretion induced by insulin and selective beta-cell necrosis *via* inducing ROS generation [37]. The CR, EEF, and AQF exhibited a slightly superior hypoglycemic effect than the STD (Metformin) possibly due to the reversal of the diabetic symptoms incurred by alloxan possibly *via* the reversal of the beta cell injury, promoting insulin secretion, sensitivity, and its action [38]. Administration of CR, EEF, and AQF antagonized weight loss due to muscle wasting obvious in the diabetic group without treatment [39], probably due to the improved insulin secretion and sensitivity coupled with enhanced glucose uptake preventing muscle wasting.

Table 2 presents the effect of the CR, EEF, and AQF on the serum AST, GGT, albumin, urea, and creatinine levels. A significant ($p < 0.05$) elevation in AST level (149.40 ± 3.47 IU/L) was exhibited by the negative control than all the groups. The CR group demonstrated significantly ($p < 0.05$) lower AST level (100.60 ± 4.12 IU/L) than the STD [(Metformin) 134.00 ± 7.31 IU/L] group. The EEF group exhibited a significant ($p < 0.05$) decrease in GGT level (4.80 ± 1.02 IU/L) than the naïve control, negative control, STD (Metformin), and CR groups. Additionally, the AQF group also demonstrated a significant ($p < 0.05$) decrease in GGT level (5.80 ± 0.80 IU/L) than the negative control (11.40 ± 0.51 IU/L) and CR (8.80 ± 0.73 IU/L) groups. However, the GGT levels of the naïve control, negative control, STD (Metformin), and CR groups weren't significantly ($p > 0.05$) different. The negative control and treated groups demonstrated a significantly ($p < 0.05$) increased albumin level than the naïve control (25.00 ± 1.58 g/L) and STD [(Metformin) 27.20 ± 0.74 g/L] groups.

All the groups exhibited significantly higher ($p < 0.05$) levels of urea than the naïve control group (6.94 ± 0.20 mM/L) except the STD [(Metformin) 7.82 ± 0.20 mM/L] group. Additionally, the EEF (12.28 ± 0.92 mM/L) and AQF (13.00 ± 1.51 mM/L) groups demonstrated significantly ($p < 0.05$) higher urea levels than the negative control (9.70 ± 0.42 mM/L), STD (Metformin), and CR (9.96 ± 0.40 mM/L) groups. Furthermore, the urea level of the CR group was significantly ($p < 0.05$) higher urea

than the negative control group. Similarly, the creatinine level of the other groups was significantly ($p < 0.05$) higher than the naïve control group except for the STD [(Metformin) 58.60 ± 7.64 μ M/L]. Additionally, the creatinine level of the negative control was significantly ($p < 0.05$) higher than STD (Metformin), EEF (71.80 ± 4.15 μ M/L), and AQF (51.40 ± 8.88 μ M/L) groups. Moreover, the creatinine level of the STD (Metformin) and AQF was significantly ($p < 0.05$) lower than the CR group.

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

The central position of the liver in metabolism makes it a vital organ in maintaining glycemic control. Liver enzymes including AST, GGT, and albumin are regarded as liver dysfunction markers with their changes reflecting the extent of liver injury [40]. A correlation between persistent hyperglycemia and increased levels of these markers was previously reported [21, 41, 42]. The elevated AST, GGT, and albumin levels in the present study might be due to the associated hyperglycemia observed (Figure 1a). However, oral administration of the CR, EEF, and AQF significantly decreased the AST and GGT levels possibly by decreasing the hyperglycemia (Figure 1a).

Urea and creatinine are normal metabolic wastes filtered out of the blood by the kidney, thus, a rise in the level of these products is regarded as a marker of kidney dysfunction and injury [43, 44]. Increased levels of creatinine and urea have been associated with impaired renal function in diabetes reflecting a decreased kidney function [44]. In our study, the urea was elevated after three weeks of treatment with decreased hyperglycemia. However, the AQF exhibited a decreased creatinine level reflecting improvement in renal function. The high level of urea and creatinine coupled with improved glycemic control observed might be due to

improved hepatic and beta cell functions without improved renal function.

The effect of the CR, EEF, and AQF on the serum electrolyte levels is displayed in Table 3. The Na⁺ level wasn't significantly different ($p > 0.05$). However, the K⁺ levels of the treated groups including STD (Metformin) were significantly ($p < 0.05$) lower than naïve (9.74 ± 0.66) and negative (9.50 ± 0.17) control groups. Moreover, both the negative control (96.00 ± 1.14) and EEF (96.60 ± 0.75) groups

demonstrated a significant ($p < 0.05$) decrease in Cl⁻ level than the naïve control (101.00 ± 1.14). Furthermore, the HCO₃⁻ level of the treated groups was significantly ($p < 0.05$) higher than the negative control (17.40 ± 0.25) group except for the AQF group (21.60 ± 1.63) which was lower than the STD [(Metformin) 26.40 ± 1.21] group. The HCO₃⁻ level of the EEF group significantly ($p < 0.05$) increased (27.40 ± 0.69) than the AQF and CR (22.20 ± 1.16) groups.

Table 3. Effects of CR, EEF, and AQF of DM on the serum levels of electrolytes

Groups	Na ⁺ (mEq/L)	K ⁺ (mEq/L)	Cl ⁻ (mM/L)	HCO ₃ ⁻ (mM/L)
Naïve	140.00 ± 1.14	9.74 ± 0.66	101.00 ± 1.14	27.60 ± 0.75
Negative control	142.60 ± 0.51	9.50 ± 0.17	96.00 ± 1.14 ^a	17.40 ± 0.25 ^a
STD (Metformin)	141.20 ± 0.84	6.36 ± 0.16 ^{ab}	97.40 ± 0.51	26.40 ± 1.21 ^e
CR	142.20 ± 1.10	6.86 ± 0.07 ^{ab}	97.60 ± 0.51	22.20 ± 1.16 ^{ae}
EEF	142.60 ± 1.34	7.32 ± 0.27 ^{ab}	96.60 ± 0.75 ^a	27.40 ± 0.69 ^{ed}
AQF	141.00 ± 0.71	7.80 ± 1.17 ^{ab}	97.80 ± 0.66	21.60 ± 1.63 ^{ac}

Values are expressed as mean ± SEM: n = 5

Table 4. Effects of CR, EEF, and AQF of DM on the Serum TC, TG, HDL-C, and LDL-C Levels

Groups	TC (mg/dl)	TG (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)
Naïve	53.82 ± 2.59 ^b	131.72 ± 11.40 ^b	97.50 ± 11.24	66.30 ± 10.68
Negative control	81.90 ± 4.45	275.90 ± 8.90	95.16 ± 10.06	47.58 ± 3.36
STD (Metformin)	46.80 ± 3.49 ^b	133.50 ± 12.59 ^b	38.22 ± 6.80 ^{ab}	25.74 ± 3.61 ^a
CR	61.62 ± 4.26 ^{bc}	165.54 ± 16.80 ^b	75.66 ± 4.20 ^c	46.80 ± 2.76
EEF	63.96 ± 2.00 ^{bc}	96.12 ± 7.67 ^{bd}	83.46 ± 7.66 ^c	38.22 ± 8.50
AQF	62.40 ± 2.76 ^{bc}	137.06 ± 4.54 ^b	78.78 ± 3.36 ^c	47.58 ± 6.00

Values are expressed as mean ± SEM: n = 5

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

An alteration in electrolyte levels observed in diabetes is due to increased blood glucose levels leading to increased plasma osmolarity and impaired renal function creating electrolyte imbalance [45]. In our study, there was no significant change in Na⁺, however, the decreased level of K⁺ observed might be induced by the treatments as some compounds might induce hypokalemia [46]. Moreover, the decreased Cl⁻ level might probably be due to the diabetic ketoacidosis-induced hyperchloremic alkalosis [47] which wasn't modified by the treatment. Additionally, the ketoacidosis might

decrease the HCO₃⁻ level in the diabetic rats, though the treatment groups exhibited significantly improved HCO₃⁻ levels except for the AQF group.

Table 4 shows the effects of CR, EEF, and AQF on the serum levels of TC, TG, HDL-C, and LDL-C. The TC level was significantly ($p < 0.05$) higher for the negative control group (81.90 ± 4.45 mg/dl) than the other groups. The treatment groups demonstrated significantly ($p < 0.05$) higher TC levels than the STD [(Metformin) 46.80 ± 3.49 mg/dl]. Similarly, observation was made for the TG of the negative control group (275.90 ± 8.90 mg/dl) than the other groups. Additionally, among the treatment groups, the EEF group demonstrated a significantly ($p < 0.05$) lower (96.12 ± 7.67 mg/dl) TG level than the CR group (165.54 ± 16.80 mg/dl). The HDL-C level of the STD (Metformin) group (38.22 ± 6.80 mg/dl)

was significantly ($p < 0.05$) decreased than the other groups. Moreover, the HDL-C level wasn't significantly different ($p > 0.05$) within the treatment, naïve control (97.50 ± 11.24 mg/dl), and negative control (95.16 ± 10.06 mg/dl) groups. Additionally, the STD (Metformin) group demonstrated a significantly ($p < 0.05$) decreased LDL-C level (25.74 ± 3.6 mg/dl) than the other groups.

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

Dyslipidemia is usually observed in diabetes and prediabetes reflecting a disturbance in lipid metabolism with accompanied elevation in TC, TG, LDL-C, and decreased HDL-C levels, though LDL-C levels might remain unchanged [48]. In our study, increased TC and TG levels without a significant ($p > 0.05$) change in HDL-C and LDL-C were observed in the diabetic rats. However, administration of CR, EEF, and AQF for three weeks significantly ($p < 0.05$) decreased the TC and TG levels without significant changes in HDL-C and LDL-C levels. The decreased TC and TG levels might reflect attenuation of dyslipidemia and improved lipid profile.

4 Conclusions

In our study, we explored the antidiabetic activity of DM leaf in alloxan-induced diabetic rats. In conclusion, DM possesses antidiabetic properties with significant hypoglycemic and hypolipidemic activity as its administration significantly lowered hyperglycemia and hyperlipidemia with improved body weight and diabetic markers levels.

5 Declarations

5.1 Acknowledgements

The author's immense gratitude goes to the Tertiary Education Trust Fund of Nigeria (TETFUND) for the research sponsorship through the Institutional Based Research and the Department of Pharmaceutical Technology, Adamawa State Polytechnic Yola for institutional support.

5.2 Author contributions

Conceptualization: M.M.D. and N.M
Investigation: M.M.D
Writing and Editing: M.M.D. and N.M

5.3 Funding

The present research was sponsored by the Tertiary Education Fund of Nigeria (TETFUND) through the Institutional-based Research Fund of Adamawa State Polytechnic, Yola.

5.4 Ethic

All animal experimental procedures were conducted according to the ethical guidelines of the Norwegian National Committee for Research Ethics in Science and Technology (NENT) 2018.

5.5 Conflict of Interest

The authors declare no conflict of interest.

6 References

- [1] American Diabetes Association Professional Practice Committee. (2022). Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes—2022. *Diabetes Care*, 45(Supplement_1), S17-S38. <https://doi.org/https://doi.org/10.2337/dc22-s002>
- [2] Athyros, V. G., Doumas, M., Imprialos, K. P., Stavropoulos, K., Georgiou, E., Katsimardou, A., & Karagiannis, A. (2018). Diabetes and lipid metabolism. *Hormones*, 17(1), 61-67. <https://doi.org/10.1007/s42000-018-0014-8>
- [3] American Diabetes Association. (2020). Pharmacologic Approaches to Glycemic Treatment: Standards of Medical Care in Diabetes—2020. *Diabetes Care*, 43(Supplement_1), S98-S110. <https://doi.org/10.2337/dc20-S009>
- [4] Dahiru, M. M., & Nadro, S. M. (2022). A review of the Mechanisms of Action and Side Effects of Anti-diabetic Agents. *Trends in Pharmaceutical Sciences*, 8(3), 195-210. <https://doi.org/http://dx.doi.org/10.30476/TI PS.2022.95931.1153>
- [5] Li, S., Odedina, S., Agwai, I., Ojengbede, O., Huo, D., & Olopade, O. I. (2020). Traditional medicine usage among adult women in Ibadan, Nigeria: a cross-sectional study. *BMC Complementary Medicine and Therapies*, 20(1), 93. <https://doi.org/10.1186/s12906-020-02881-z>

- [6] Verma, S., Gupta, M., Popli, H., & Aggarwal, G. (2018). Diabetes mellitus treatment using herbal drugs. *International Journal of Phytomedicine*, 10(1), 1-10.
- [7] Bindu, J., & Narendhirakannan, R. T. (2018). Role of medicinal plants in the management of diabetes mellitus: a review. *3 Biotech*, 9(1), 4. <https://doi.org/10.1007/s13205-018-1528-0>
- [8] Dahiru, M. M. (2023). Recent advances in the therapeutic potential phytochemicals in managing diabetes. *Journal of Clinical and Basic Research*, 7(1), 13-20. <https://doi.org/http://dx.doi.org/10.61186/jcbr.7.1.13>
- [9] Dahiru, M. M., Umar, A. S., Muhammad, M., Fari, I. I., & Musa, Z. Y. (2024). Phytoconstituents, Fourier-Transform Infrared Characterization, and Antioxidant Potential of Ethyl Acetate Extract of *Corchorus olitorius* (Malvaceae). *Sciences of Phytochemistry*, 3(1), 1-10. <https://doi.org/https://doi.org/10.58920/sciph0301208>
- [10] Dahiru, M. M., & Nadro, M. S. (2022). Phytochemical Composition and Antioxidant Potential of *Hyphaene thebaica* Fruit. *Borneo Journal of Pharmacy*, 5(4), 325-333. <https://doi.org/https://doi.org/10.33084/bjop.v5i4.3632>
- [11] Dahiru, M. M., & Nadro, S. M. (2022). Hypolipidemic Potential of Ethyl acetate Extract of *Hyphaene thebaica* Fruit in Streptozotocin-induced Diabetic Rats. *Majalah Obat Tradisional*, 27(2), 159-164. <https://doi.org/https://doi.org/10.22146/mot.75401>
- [12] Dahiru, M. M., Ahmadi, H., Faruk, M. U., Aminu, H., Hamman, & Abreme, G. C. (2023). Phytochemical Analysis and Antioxidant Potential of Ethylacetate Extract of *Tamarindus Indica* (Tamarind) Leaves by Frap Assay. *Journal of Fundamental and Applied Pharmaceutical Science*, 3(2), 45-53. <https://doi.org/https://doi.org/10.18196/jfaps.v3i2.16708>
- [13] Musa, N., Dahiru, M. M., & Badgal, E. B. (2024). Characterization, In Silico Antimalarial, Antiinflammatory, Antioxidant, and ADMET Assessment of *Neonauca excelsa* Merr. *Sciences of Pharmacy*, 3(2), 92-107. <https://doi.org/https://doi.org/10.58920/sciphar0302232>
- [14] Doughari, J. H. (2012). *Phytochemicals: extraction methods, basic structures and mode of action as potential chemotherapeutic agents*. INTECH Open Access Publisher Rijeka, Croatia.
- [15] Uduwana, S., Abeynayake, N., & Wickramasinghe, I. (2023). Synergistic, antagonistic, and additive effects on the resultant antioxidant activity in infusions of green tea with bee honey and Citrus limonum extract as additives. *Journal of Agriculture and Food Research*, 12, 100571. <https://doi.org/https://doi.org/10.1016/j.jafr.2023.100571>
- [16] Dahiru, M. M., James, D., Abdulhasib Oluwatobi, O., Hesper Alex, Z., Rejoice Daniel, P., Usanye, Z., Patience, C., Hauwa Yahaya, A., & Muhammad, Z. (2024). Phytoconstituents and In Vitro Free Radical Scavenging Potential of n-Hexane and Aqueous Fractions of *Cucurbita maxima* and *Leptadenia hastata*. *Sciences of Pharmacy*, 3(4), 193-202. <https://doi.org/https://doi.org/10.58920/sciphar0304265>
- [17] Dahiru, M. M., & Musa, N. (2024). Phytochemical Profiling, Antioxidant, Antidiabetic, and ADMET Study of *Diospyros mespiliformis* Leaf, Hochst Ex A. Dc Ebenaceae. *J. Fac. Pharm. Ankara/Ankara Ecz. Fak. Derg*, 48(2), 412-435. <https://doi.org/http://dx.doi.org/10.33483/jfpau.1354293>
- [18] Dahiru, M. M., Musa, N., Abaka, A. M., & Abubakar, M. A. (2023). Potential Antidiabetic Compounds from *Anogeissus leiocarpus*: Molecular Docking, Molecular Dynamic Simulation, and ADMET Studies. *Borneo Journal of Pharmacy*, 6(3), 249-277. <https://doi.org/https://doi.org/10.33084/bjop.v6i3.5027>
- [19] Dahiru, M. M., & Musa, N. (2024). GC-MS Analysis, Antioxidant, Antidiabetic Activity, and ADMET Study of *Diospyros mespiliformis* Hochst. Ex A. DC. Ebenaceae Stembark. *Hacettepe University Journal of the Faculty of Pharmacy*, 44(3), 198-219.
- [20] Dahiru, M. M., & Musa, N. (2024). Antidiabetic Activity of *Diospyros mespiliformis* on Alloxan-Induced Diabetic Rats. *Journal of Fundamental and Applied Pharmaceutical Science*, 5(1), 11-20.
- [21] Dahiru, M. M., & Nadro, M. S. (2022). Anti-diabetic potential of *Hyphaene thebaica* fruit in streptozotocin-induced diabetic rats. *Journal of Experimental and Molecular Biology*, 23(1), 29-36. <https://doi.org/https://doi.org/10.47743/jemb-2022-63>
- [22] Dahiru, M. M., Alfa, M. B., Abubakar, M. A., & Abdullahi, A. P. (2024). Assessment of in silico antioxidant, anti-inflammatory, and antidiabetic activities of *Ximenia americana* L. Olacaceae. *Advances in Medical, Pharmaceutical and Dental Research*, 4(1), 1-13.

- <https://doi.org/http://dx.doi.org/10.21622/A-MPDR.2024.04.1.735>
- [23] Alkhalaf, M. I., Hussein, R. H., & Hamza, A. (2020). Green synthesis of silver nanoparticles by *Nigella sativa* extract alleviates diabetic neuropathy through anti-inflammatory and antioxidant effects. *Saudi Journal of Biological Sciences*, 27(9), 2410-2419.
- [24] Dahiru, M. M., Ahmadi, H., Faruk, M. U., Aminu, H., & Hamman, A. G. C. (2023). Phytochemical Analysis and Antioxidant Potential of Ethylacetate Extract of *Tamarindus Indica* (Tamarind) Leaves by Frap Assay. *Journal of Fundamental and Applied Pharmaceutical Science*, 3(2), 45-53.
- [25] Aminu, S. A., Ibrahim, Y., Ismail, H. A., & Ibrahim, I. O. (2021). Medicinal and Traditional Utilization of African Ebony (*Diospyros mespiliformis*): A Review. *International Journal of Current Microbiology and Applied Sciences*, 10(06), 811-817.
- [26] Norwegian National Research Ethics Committees. (2018). *Ethical Guidelines for the Use of Animals in Research*. The Norwegian National Committee for Research Ethics in Science and Technology. <https://www.forskningsetikk.no/en/guidelines/science-and-technology/ethical-guidelines-for-the-use-of-animals-in-research/>
- [27] Ighodaro, O. M., Adeosun, A. M., & Akinloye, O. A. (2017). Alloxan-induced diabetes, a common model for evaluating the glycemic-control potential of therapeutic compounds and plants extracts in experimental studies. *Medicina*, 53(6), 365-374. <https://doi.org/https://doi.org/10.1016/j.medic.2018.02.001>
- [28] Reitmann, S. (1957). Colorimetric method for the determination of serum glutamic pyruvate and glutamic oxaloacetate transaminase. *Amer. J. Clin. Path.*, 28, 56.
- [29] Szasz, G. (1969). A kinetic photometric method for serum γ -glutamyl transpeptidase. *Clinical Chemistry*, 15(2), 124-136.
- [30] Grant, G. H. (1987). *Amino acids and proteins (Fundamentals of clinical chemistry)* (3th edition ed.). WB Saunders Company.
- [31] Chaney, A. L., & Marbach, E. P. (1962). Modified reagents for determination of urea and ammonia. *Clinical Chemistry*, 8(2), 130-132.
- [32] Bartels, H., Böhmer, M., & Heierli, C. (1972). Serum creatinine determination without protein precipitation. *Clinica chimica acta; international journal of clinical chemistry*, 37, 193-197.
- [33] Stein, E. A. (1987). *Lipids, lipoproteins and apolipoproteins* (3rd Edn. ed.). W.B Saunders
- [34] McGowan, M. W., Artiss, J. D., Strandbergh, D. R., & Zak, B. (1983). A peroxidase-coupled method for the colorimetric determination of serum triglycerides. *Clinical Chemistry*, 29(3), 538-542.
- [35] Warnick, G. R., & Albers, J. (1978). A comprehensive evaluation of the heparin-manganese precipitation procedure for estimating high density lipoprotein cholesterol. *Journal of Lipid Research*, 19(1), 65-76.
- [36] Friedewald, W. T., Levy, R. I., & Fredrickson, D. S. (1972). Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clinical Chemistry*, 18(6), 499-502.
- [37] Lenzen, S. (2008). The mechanisms of alloxan- and streptozotocin-induced diabetes. *Diabetologia*, 51(2), 216-226. <https://doi.org/10.1007/s00125-007-0886-7>
- [38] Li, G. Q., Kam, A., Wong, K. H., Zhou, X., Omar, E. A., Alqahtani, A., Li, K. M., Razmovski-Naumovski, V., & Chan, K. (2012). Herbal medicines for the management of diabetes. *Advances in experimental medicine and biology*, 771, 396-413. https://doi.org/10.1007/978-1-4614-5441-0_28
- [39] Perry, B. D., Caldwell, M. K., Brennan-Speranza, T. C., Sbaraglia, M., Jerums, G., Garnham, A., Wong, C., Levinger, P., ul Haq, M. A., & Hare, D. L. (2016). Muscle atrophy in patients with Type 2 Diabetes Mellitus: roles of inflammatory pathways, physical activity and exercise. *Exercise Immunology Review*, 22, 94.
- [40] De Silva, N. M. G., Borges, M. C., Hingorani, A. D., Engmann, J., Shah, T., Zhang, X., Luan, J. a., Langenberg, C., Wong, A., & Kuh, D. (2019). Liver function and risk of type 2 diabetes: bidirectional Mendelian randomization study. *Diabetes*, 68(8), 1681-1691.
- [41] Wan, J.-Y., & Yang, L.-Z. (2022). Liver enzymes are associated with hyperglycemia in diabetes: A three-year retrospective study. *Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy*, 545-555.
- [42] Islam, S., Rahman, S., Haque, T., Sumon, A. H., Ahmed, A. Z. M., & Ali, N. (2020). Prevalence of elevated liver enzymes and its association with type 2 diabetes: A cross-sectional study in Bangladeshi adults. *Endocrinology, diabetes & metabolism*, 3(2), e00116.
- [43] Higgins, C. (2016). Urea and the clinical value of measuring blood urea concentration. *Acutecaretesting. Org*, 1-6.
- [44] Kamal, A. (2014). Impact of diabetes on renal function parameters. *Ind J Fund Appl Life Sci*, 4(3), 411-416.
- [45] Eshetu, B., Worede, A., Fentie, A., Chane, E., Fetene, G., Wondifraw, H., Shimelis, M., Girma,

- M., Hadgu, R., & Demeke, K. (2023). Assessment of Electrolyte Imbalance and Associated Factors Among Adult Diabetic Patients Attending the University of Gondar Comprehensive Specialized Hospital, Ethiopia: A Comparative Cross-Sectional Study. *Diabetes, Metabolic Syndrome and Obesity*, 1207-1220.
- [46] Veltri, K., & Mason, C. (2015). Medication-Induced Hypokalemia. *P & T : a peer-reviewed journal for formulary management*, 40, 185-190.
- [47] Yasuda, K., Hayashi, M., Murayama, M., & Yamakita, N. (2016). Acidosis-Induced Hypochloremic Alkalosis in Diabetic Ketoacidosis Confirmed by The Modified Base Excess Method. *The Journal of Clinical Endocrinology & Metabolism*, 101(6), 2390-2395. <https://doi.org/10.1210/jc.2016-1324>
- [48] Bhowmik, B., Siddiquee, T., Mujumder, A., Afsana, F., Ahmed, T., Mdala, I. A., do V. Moreira, N. C., Khan, A. K. A., Hussain, A., & Holmboe-Ottesen, G. (2018). Serum lipid profile and its association with diabetes and prediabetes in a rural Bangladeshi population. *International journal of environmental research and public health*, 15(9), 1944.