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Hypolipidemic Potential of Ethyl Acetate Extract of *Hyphaene thebaica* Fruit in Streptozotocin-induced Diabetic Rats

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ABSTRACT

Hyperlipidemia is characterized by elevated levels of Triglycerides, Cholesterol, LDL, and decreased levels of HDL are challenges in the management of Diabetes mellitus which might lead to death. This study aimed to determine the hypolipidemic potential of ethyl acetate extract of *Hyphaene thebaica* fruit in streptozotocin-induced diabetic rats. The hypolipidemic potential of *H. thebaica* fruit in streptozotocin-induced diabetic rats was determined. Rats were divided into six groups. Treatment groups were administered the extract at doses of 400 and 200 mg/kg body weight. Oral administration of the extract at 400 mg/kg body weight for four weeks significantly ($p < 0.05$) decreased the levels of total cholesterol (151.7 mg/dl ±2.40), Triglyceride (84.0 mg/dl ±1.15), LDL (75.2 mg/dl ±1.20) and increased HDL (39.3 mg/dl ±1.88) level. However no significant ($p < 0.05$) difference was observed between the 400 and 200 mg/kg body weight dose. Conclusively, *H. thebaica* can be used as a medicinal plant due to its protective action against dyslipidemic complications of diabetes mellitus.
Keywords: Hypolipidemic; Doum Palm; Dyslipidemia; Diabetes

INTRODUCTION

Diabetes mellitus is a state of impaired carbohydrate metabolism disorders characterized and identified by the presence of hyperglycemia without treatment subsequently causing secondary changes in water/electrolyte balance, protein, and lipid metabolism (American Diabetes Association [ADA], 2021). The long-term specific effects of diabetes include retinopathy, nephropathy, and neuropathy, among other complications (World Health Organization [WHO], 2019). Insulin directs the uptake of glucose by cells of the body specifically muscles and adipose tissues. However, in its absence, the cells are unable to take up glucose and utilize it properly (Victor *et al.,* 2018). In the other scenario where there is resistance, insulin is available, however, the tissues are insensitive to its action.

Decreased production of insulin which is usually caused by autoimmune destruction of the beta-cells of Langerhans, leads to type 1 diabetes (insulin-dependent diabetes). Insulin-dependent diabetes is characterized by a hyperglycemia condition due to decrease glucose uptake by cells and increased glucose production by the liver. Therefore type 1 diabetes leads to dependence on an exogenous source of insulin (Victor *et al.,* 2018). In type 2 diabetes (non-insulin-dependent), there is decreased production or increased demand for insulin due to the resistance of insulin receptors or a decrease in the number of the receptors on the target tissue leading to hyperglycemia (Victor *et al.,* 2018).

Worldwide, an estimated 9.3% of adults aged between 20-79 years, which is a staggering 463 million people are living with diabetes globally, which is expected to rise to 578 million in 2030 (International Diabetes Federation [IDF], 2019). The increased incidence and prevalence of type 2 diabetes in the aging developed world is partly due to sedentary lifestyles and obesity with the incidence in children being more disturbing (Denise, 2017). Symptoms of diabetes mellitus include glycosuria (which might present without symptoms), polyuria, polydipsia, and polyphagia, accompanied by loss of weight (Victor *et al.,* 2018).

Vascular complications categorized into microangiopathy and macroangiopathy observed in diabetes can lead to death due to atherosclerosis lesions which form more rapidly than in non-diabetes patients (Decroli, 2019). Microvascular complications in diabetes are neuropathy, nephropathy, and retinopathy; while macrovascular complications are coronary artery disease, peripheral artery disease, artery renal sclerosis, and even stroke (Lofty *et al.,* 2017). Disturbance in lipid metabolism contributes to the formation of atherosclerosis lesions marked by decreased levels of high-density lipoprotein (HDL), elevated low-density lipoprotein (LDL), and rising Triglyceride (TG) (Ozder, 2014). Thus, the need for

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the detection of dyslipidemia and its treatment to reduce the cardiovascular risk and its consequences in diabetic patients.

Though oxidative stress is one of the challenging complications of diabetes mellitus, many medicinal plants are employed in the management of the disease. These plants are made up of antioxidants such as phenols and flavonoid compounds which scavenge free radicals and lower the oxidative stress caused by such free radicals (Mohd *et al.,* 2017). Some of the medicinal plants used in the management of diabetes include *Coccinia indica, Moringa oleifera, Eugenia jambolana, Tinospora cordifolia, Zingiber officinale, Aegle marmelos, Cinnamomum tamala, Trichosanthes cucumerina, Leptadania hastata, Anisopus mannii, Opium sanctum, Hyphaene thebaica* Han *et al.*, 2019).

Hyphaene thebaica (Doum palm fruit) is a desert palm tree with edible oval fruit native to the Nile valley, which is a member of the palm family, Arecaceae, and a source of potent antioxidants (Ghada *et al.,* 2020). Doum palm fruit have significant antimicrobial activities which were attributed to the presence of flavonoids. Also, the aqueous extract of doum fruits showed an antioxidant activity; this is due to the substantial amount of their water-soluble phenolic contents (Ghada *et al.,* 2020). *Hyphaene thebaica* fruit also has hypolipidemic activity as the administration of the fruit decoction significantly lowers blood cholesterol, glucose, triglycerides, and total lipids (Bayad, 2016).

In this context, this study aimed to evaluate the hypolipidemic potential of *Hyphaene thebaica* fruit in streptozotocin-induced diabetic rats.

METHODOLOGY

Materials

Hyphaene thebaica fruit was collected from Mayo-belwa Local Government Area of Adamawa State, Nigeria. The plant was authenticated by a Botanist with the Department of Plant Sciences, Modibbo Adama University Yola. The fruit pulp was dried and the mesocarp was removed and ground into powder using mortar and pestle. Streptozotocin (STZ) Powder (InvivoChem, USA), was used for induction of diabetes while Metformin**:** Diabetmin® tablets (Hovid Pharmaceuticals Ltd, Nigeria) was used as a reference drug. Cholesterol, triglycerides, LDL, HDL Kits (RANDOX Laboratories, Antrim, UK). All other chemicals and reagents were of AnarlaR.

Microliter pipette: Witopet Premium (Witeg Labortecnik GmbH, Germany), Incubator/oven: UNISCOPE SM9053, and Spectrophotometer: 556 (Surgifriend Medicals, England), Centrifuge: 80-2 (Techmel and Techmel, USA), Portable glucometer: SD CodeFree™ (SD Biosensor, Inc., Korea), Centrifuge [Spectrafuge™ (Corning Life Sciences, USA)]. Male Wistar albino rats weighing 172 g ±10 were used for the study.

The rats were obtained from the Animal House of Gombe State University, Nigeria. They were maintained under a standard condition of light (12-hour light) and fed a standard diet (Finisher pellet, ECWA feed Nigeria Ltd, Jos) and water *ad libitum.* All animal experimental procedures were conducted according to the ethical guidelines of the National Committee for Research Ethics in Science and Technology (NENT) 2018, (NNREC, 2019).

Methods

Extract Preparation

H. thebaica fruit pulp powder (500 g) was macerated with 1.5 L ethyl acetate in a glass jar for 2 days at room temperature. The extract was filtered and concentrated to dryness under reduced pressure at 40°C (Trease and Evans, 2009).

Induction of diabetes

The rats were fasted overnight and diabetes was induced by a single intraperitoneal injection of a freshly prepared solution of STZ (50 mg/Kg body weight) in normal saline. The rats were allowed to drink 5% glucose solution overnight to overcome the drug-induced hypoglycemia. Rats with blood glucose levels >200 mg/dl were considered diabetic (Al-Hariri, 2012).

Experimental design

The rats were divided into six groups and treated daily by intragastric tube for four weeks.
Group 1: Normal (control group); Group 2: Normal rats treated with 400 mg/kg body weight of Ethyl acetate extract of *H. thebaica* fruit; Group 3: rats injected with STZ without treatment (STZ-induced diabetic group); Group 4*:* STZ-induced diabetic rat treated with 80 mg/kg body weight Metformin; Group 5: STZ-induced diabetic rats treated with 400 mg/Kg body weight of Ethyl acetate extract of *H. thebaica* fruit; Group 6: STZ-induced diabetic rats treated with 200 mg/Kg body weight Ethylacetate extract of *H. thebaica* fruit.

Lipid profile

Blood samples were collected from the heart through cardiac puncture and centrifuge at 3000 rpm for 15 minutes to separate serum from cells in order to analyze for total cholesterol (TC), Triglyceride (TG), High-density Lipoprotein (HDL), and Low-density Lipoproteins (LDL). The

measurement of TC, TG, HDL, and LDL was used to assess the lipid profile.

Determination of Total cholesterol (TC)

The serum level of total cholesterol was quantified after enzymatic hydrolysis and oxidation of the sample according to the method of Stein (1987). A number of 100 µL of the reagent was added to each of the samples and standard, incubated for 10 minutes at 25^0C after mixing. The absorbance of the sample and standard was measured spectrophotometrically within 30 min as reaction time at 546 nm. The value of total cholesterol present in the serum was expressed in mmol/L.

Determination of Triglyceride concentration (TG)

Ten µL of distilled water, standard, and the sample were dispensed into standard and sample test tubes respectively. 100 µL of working reagent (reagent 1) was added to each test tube, mixed, and incubated for 5 minutes at 37^0C. Absorbance was measured at 500 nm (Tietz, 1990).

Determination of High-density lipoprotein (HDL)

The serum level of HDL was measured using the method of Wacnic and Albers (1978). Low-density lipoproteins (LDL), very-low-density lipoproteins (VLDL), and chylomicron fractions in the sample were precipitated quantitatively by the addition of phosphotungstic acid in the presence of magnesium ions. The mixture was allowed to stand for 10 minutes at room temperature (20-25^0C) and centrifuged for 10 minutes at 1200*g.* The supernatant represents the HDL fraction Wacnic and Albers (1978).

Determination of Low-density lipoprotein cholesterol (LDL)

The serum level of LDL was measured according to the protocol of Friedewald *et al*. (1972) using the equation below:

$$\text{LDL cholestrol} = \text{Total cholestrol} - \text{HDL cholestrol} - \frac{\text{Triglyceride}}{2.2}$$

Statistical analysis

Data were expressed as mean ± standard error of mean (± SEM). Differences among treatment group means were assessed by one-way analysis of variance (ANOVA) followed by the Tukey multiple comparison test. Group means were considered to be significantly different at $p < 0.05$. Data were statistically evaluated using Statistical Package for the Social Sciences (SPSS) version 22 Software.

RESULTS AND DISCUSSION

The result of the effect of ethyl acetate extract of *H. thebaica* fruit on TC, TG, HDL, and LDL levels in STZ-induced diabetic rats is presented in Table I. The result revealed that the negative control (191.7 mg/dl ±2.33) had a significantly ($p < 0.05$) higher Total Cholesterol (TC) than all the other groups. The extracts groups and standard control had a significantly ($p < 0.05$) higher TC compared to normal and baseline control.

Triglycerides (TG) level was observed to be significantly ($p < 0.05$) higher in the negative control (115.7 mg/dl ±2.08) than in all the other groups. The extracts groups and standard control had a significantly ($p < 0.05$) higher TG compared to normal and baseline control. The High-density lipoprotein (HDL) level of the negative control (34.3 mg/dl ±1.58) was significantly ($p < 0.05$) lower than all the groups. The extracts groups and standard control had a significantly ($p < 0.05$) higher TC compared to normal and baseline control. The result of the low-density lipoprotein (LDL) revealed that the negative control (104.7 mg/dl ±2.88) had a significantly ($p < 0.05$) higher LDL compared to all other groups. The standard control and extracts groups were significantly higher than the normal (38.3 mg/dl ±1.15) and baseline (40.6 mg/dl ±1.45) control.

Diabetes mellitus is associated with a disturbance in lipid metabolism characterized by increased levels of triglycerides, LDL, cholesterol, and decreased HDL levels (Schofield *et al.,* 2016). In another study, herbal medicines were shown to be capable of preventing heart and vascular disease frequently observed in complications of diabetes (Edgar *et al.,* 2018). In the present study, oral administration of ethyl acetate extract of *H. thebaica* at a dose of 200 and 400 mg/kg b. wt. led to significant improvement in the lipid profile of diabetic rats. This might be due to the ability of the extract to normalize the lipid profile by inhibiting cholesterol biosynthesis, absorption, and modifying the activity of lipogenic and lipolytic enzymes, leading to reduced lipid metabolism (Salah *et al.,* 2011).

The level of cholesterol in the blood is related to the development of atherosclerosis and myocardial infarction. In diabetes mellitus, the acetyl CoA pool is increased and more molecules are channeled to cholesterol, thus abnormality of cholesterol metabolism may lead to cardiovascular accidents and heart attacks associated with DM (Vasudevan *et al.,* 2018). The reduction of cholesterol may not only decrease the lipid content of the plaque but can also reduce the accumulation of monocytes and macrophages (Satyanarayana

Table I. Effect of Ethylacetate Mesocarp Extract of *H. thebaica* Fruit on TC, TG, HDL, and LDL levels in STZ-induced diabetic rats

Groups (Treatments per Kg b. wt.)	Lipid parameters (mg/dl)			
	TC	TG	HDL	LDL
1 (Normal)	115.0 ±1.73^{cde}	66.0 ±1.73^{cde}	46.7 ±1.45	38.3 ±1.15^{cde}
2 (Normal + 400 mg extract)	116.3 ±2.19^{cde}	65.3 ±2.03^{cde}	46.0 ±0.88	40.6 ±1.45^{cde}
3 (Diabetic without treatment)	191.7 ±2.33	115.7 ±2.08	34.3 ±1.58^{abde}	104.7 ±2.88
4 (Diabetic + 80 mg Metformin)	145.4 ±1.20^{c}	85.1 ±1.20^{c}	42.7 ±1.45^{ab}	59.0 ±1.28^{c}
5 (Diabetic + 400 mg extract)	151.7 ±2.40^{c}	84.0 ±1.15^{c}	39.3 ±1.88^{ab}	75.2 ±1.20^{c}
6 (Diabetic + 200 mg extract)	155.4 ±2.90^{c}	86.7 ±0.88^{c}	38.7 ±1.76^{ab}	75.3 ±0.88^{c}

TC= Total cholesterol; TG= Triglycerides; HDL= High-density lipoproteins; LDL= Low-density lipoprotein; Values are expressed as mean ± SEM: n = 5; Values in the same column with ^{a} superscript were significantly ($p < 0.05$) lower than the Normal control; Values in the same column with ^{b} superscript were significantly ($p < 0.05$) lower than Baseline control; Values in the same column with ^{c} superscript were significantly ($p < 0.05$) lower than the Negative control; Values in the same column with ^{d} superscript were significantly ($p < 0.05$) lower than the Standard (Metformin) control; Values in the same column with ^{e} superscript were significantly ($p < 0.05$) lower than Groups 5 and 6.

and Chakrapani, 2019). In this study, an increased level of cholesterol was observed after four weeks which might be due to the enhancement in the biosynthesis of the cholesterol contents and the reduction of absorption of the cholesterol contents (Jayaraman *et al.,* 2018).

Increased lipid metabolism is observed in diabetes due to insulin deficiency resulting from damage to pancreatic cells. Increased hyperlipidemia observed in the diabetic rats was due to the absence of insulin which inhibits hormone-sensitive lipase, thus increased and continuous activity of lipolytic enzymes is observed leading to an increase in the level of cholesterol, triglycerides, LDL and decrease in HDL (Victor *et al.,* 2018). Oral administration of flavonoid extracts to diabetic rats significantly increased adiponectin levels speculated to be important in the regulation of lipid metabolism (Salah *et al.,* 2011). Thus, the improvement of the lipid profile observed in the present study might be due to the presence of flavonoids in the extracts due to their properties of inhibiting cholesterol biosynthesis and absorption and modifying the activity of lipogenic and lipolytic enzymes, leading to reduced lipid metabolism. In another study, saponins were also shown to decrease the serum level of cholesterol (Patel *et al.,* 2012). In the present study oral administration *of H. thebaica* led to a significant decrease in the level of cholesterol which might be due to the presence of flavonoids and saponins present in the extract.

This study agrees with the report of Tohamy *et al.,* (2013), of a significant ($p < 0.05$) increase in the level of cholesterol, triglycerides, LDL and decrease in HDL after oral administration of aqueous mesocarp extract of *H. thebaica* at the dose of 1 g/Kg b. wt. for four weeks. The present study agrees with the report of El Halim (2020), where elevated levels of total lipid, triglyceride, total cholesterol, and LDL in diabetic rats were observed compared with normal control rats. However, treatment with *H. thebaica* extracts significantly ($P < 0.05$) decreased the total lipids, triglyceride, total cholesterol, and LDL of all treated groups as compared to the diabetic rats. Abdulazeez *et al.,* (2019) and Bayad (2016), reported results similar to the present study.

CONCLUSION

Oral administration of *H. thebaica* also showed an improvement in the lipid profile of diabetic rats which shows its protective action against dyslipidemic complications associated with diabetes mellitus.

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