Original Research Article

HYPOGLYCEMIC AND HYPOLIPIDEMIC EFFECT OF *Dialum guineense* (ICHEKU) FRESH LEAVES ON ALLOXAN-INDUCED DIABETES IN MALE ALBINO RATS

ABSTRACT

Background: According to current Globe Health Organization predictions, the number of diabetes patients globally will reach 370 million by 2030. It is expected to become one of the most frequent human degenerative diseases, necessitating immediate attention. Plants have long been used as a primary source of medicine.
Purpose: This study evaluated the effects of *Dialium guineense* (Dg) ethanol extract on hypoglycemic and hypolipidemic properties of alloxan-induced albino rats
Methods: Thirty-six (36) healthy male adult albino rats weighing 125-150g were divided into six groups with six rats each. Group 1 was the normal control (received feed and water only), and group 2 received only alloxan and served as untreated diabetic rats. Group 3 received 5 mg/kg bw of glibenclamide (standard drug), while groups 4, 5, and 6 received 200 mg/kg bw, 500 mg/kg bw, and 1000 mg/kg bw of Dg extract, respectively, for 28 days. The rats were induced intraperitoneally using alloxan monohydrate (100 mg/kg bodyweight). At the end of the experiment, the rats were sacrificed under mild anesthesia with chloroform. Blood was collected for biochemical evaluation using standard techniques (Randox kits). Fasting blood glucose level was checked weekly.
Result: Blood sugar levels in the diabetic control group experienced severe hyperglycemia compared to normal animals. The blood glucose level in the negative control group was 359.5 ± 1.6 mg/dl on Day 0 and 389.0 ± 1.5 mg/dl on Day 28. Administration of *Dialum guineense* extract at 200 mg/kg, 500 mg/kg, and 1000 mg/kg significantly ($P < 0.05$) decreased the fasting blood glucose level in the diabetic rats on days 7,14,21, and 28 as compared to the negative control group. Increased total cholesterol (TC), triglyceride, and low-density lipoprotein cholesterol (LDL-C) activities reduced significantly ($p<0.05$) in both groups treated with ethanol extract of *Dialum guineense* when compared with untreated diabetic rats. In contrast, HDL-C was not statistically significant compared to untreated diabetic rats.
Conclusion: These findings suggest that ethanolic extract of *Dialum guineense* leaves may have hypoglycemic and hyperlipidemic properties and may be used in managing diabetes, whose pathogenesis and progression are known to be influenced by oxidant species.

KEYWORDS: *Alloxan monohydrate; Diabetes, Dialum guineense, Glibenclamide, Lipid Profile.*

INTRODUCTION

Medicinal plants have been utilized as a source of medicine in practically all societies from time immemorial [1]. Because of the huge development of medicine and a growing interest in herbal therapies, the usage of medicinal plants is on the rise all over the world. Medicine uses plants to maintain and improve physical, mental, and spiritual well-being and cure specific illnesses and disorders [2]. Medicinal plants are precious gifts from Mother Nature, who has preserved such treatments in her plant world for

people to employ in their fights against sickness and cures. It is up to us to go out and look for these gems, investigate, search for them, and gain from them.

Dialium guineense (Velvet tamarind) is one of the promising plants of interest. It is a medicinal plant belonging to the Fabaceae family and the Caesalpinioideae subfamily. In folklore medicine, various portions of the tree have been utilized to cure various maladies, including cancer, headaches, and pains. The bark is used for oral hygiene and stomach discomfort among the Esan tribe of the Edo state. According to Idu et al. [3], the leaves are used for fever, pregnancy pains, and edema; and the fruits are used for diarrhea [4]. The fruit's pulp is edible and sweet, with relatively low quantities of ascorbic acid and tannin. It contains a lot of protein and minerals [5].

Previous research has revealed that the plant contains saponins, which are thought to aid in tooth cleaning while also preventing caries and plaque [6]. *D. guineense* is used as an antiulcer and a vitamin supplement by some tribes in the southern portion of Nigeria, according to Lawal et al. [7]. It has traditionally been used to treat heart problems. However, Lawrence et al. [8] found that the tannins component had strong cardiac preventive properties and antioxidant action. It causes lipoprotein, which transports cholesterol, to precipitate, lowering the amount of cholesterol ingested.

Diabetes mellitus (DM) is a metabolic and life-threatening condition affecting people worldwide [9]. It is a chronic condition characterized by abnormalities in glucose, protein, and lipid metabolism resulting from absolute or inadequate insulin insufficiency [10,11]. DM is characterized by physiological and cellular abnormalities that lead to the death of beta (β) cells as the illness progresses [12]. β-cell failure is caused by glucose and lipid toxicity, and increased glucose absorption by the islet beta (β)-cells induces glucose toxicity [12]. Diabetes mellitus increases the likelihood of developing several other illnesses, many of which are severe and can ultimately lead to death [11]. Nephropathy, neuropathy, retinopathy, and angiopathy are some disorders that might develop in diabetic individuals [11].

Increased sugar levels cause glycation and electron transport chain reactions, resulting in an imbalance in the cell's antioxidant capacity due to increased reactive oxygen species generation [12]. The oxidative stress that results from the process causes a reduction in insulin synthesis and secretion, which sets off a chain of cellular events that eventually leads to death [12]. Anomalies in serum lipids have also been linked to diabetes [13]. High levels of total cholesterol, triglycerides, low-density lipoprotein cholesterol

(LDL-C), and low levels of high-density lipoprotein cholesterol (HDL-C) have previously been associated with coronary heart disease [13]. Current treatment strategies that employ chemo-drugs such as metformin and sulfonylurea have dissipated multidrug resistance and other adverse effects, such as gastrointestinal symptoms, fluid body buildup, and heart disease [14]. As a result, there is a need to find highly effective, safe, and harmless sugar-level oral pharmaceuticals that are free of the negative effects associated with prior oral medications used to treat diabetes [15].

Medicinal herbs have been used to cure diabetes, but only a handful have been properly studied [10], and oral antidiabetic medicines have a high rate of treatment failures and severe side effects, necessitating the urgent need for alternative therapies [16]. Despite all of the research on this plant, little is known about its hypoglycemic and hypolipidemic activity, so the current study was undertaken to investigate the hypoglycemic and hypolipidemic effects of *Dialum guineense* to establish the pharmacological basis for its traditional use to treat diabetes and some underlying complications.

MATERIALS AND METHODS

Plant Materials

Dialum guineense (Icheku) leaves were gathered from Lodu Ndume farmland in Umuahia North Local Government of Abia State. The leaves were collected, washed, and dried at room temperature; it was weighed and milled into powdered form 250g. The powdered leaves were soaked in Ethanol and distilled water in the ratio of 80:20 respectively and left to stand for 3 days with occasional shaking. This was filtered using Whatman No.1 filter paper, and the filtrate was evaporated to obtain the dry matter; a rotary evaporator under reduced pressure at 40ºC was used.

Experimental Animals

Thirty-six (36) healthy adult albino rats weighing 125-150g procured from the College of Veterinary Medicine, the Michael Okpara University of Agriculture, Umudike, Abia State, were used for the study. The animals, on arrival, were weighed to obtain initial weight and were acclimatized for 14 days in the animal house of the Biochemistry Department, College of Natural Sciences, Michael Okpara University of Agriculture, Umudike. The animals were allowed access to standard food and water until the research's end, which lasted for 28 days.

Induction of Hyperglycemia

Experimental diabetes was established by injecting 100 mg of newly made alloxan monohydrate diluted in 10 ml of normal saline in a single dosage, equivalent to l00 mg/kg bw of alloxan. After determining the baseline test, it was delivered intraperitoneally according to body weight within a few minutes of preparation. Blood was obtained three days later to chemically establish the diabetes status by cutting a tail end incision with sharp scissors and a reagent strip. The rats' fasting blood glucose levels were measured with a portable glucometer, and their blood glucose levels were more than 185mg/dl after 4 days post-induction.

Experimental Design and Animal Grouping

Rats were divided into six groups of six rats each. Glucose level was observed on days 0, 7th, 14th, 21st, and 28th

Chart 1 : Group wise Treatment criteria

Group		Treatment
1	Normal Control	Feed + H_2O ad libitum
2	Negative control	Alloxan + feed+ H_2O ad libitum
3	Positive control	Alloxan + standard drug (Glibenclamide) +feed+ H_2O
4	Diabetic treated rats	Alloxan + 200ml/kg Honey + feed+ H_2O ad libitum
5	Diabetic treated rats	Alloxan + 500ml/kg honey + feed+ H_2O ad libitum
6	Diabetic treated rats	Alloxan + 1000ml/kg honey + feed+ H_2O ad libitum

Sacrifice and Sample Collection

After the experiment, the rats were sacrificed under mild anesthesia with chloroform. Blood samples were collected into lithium heparin bottles for biochemical analysis.

Biochemical metabolic parameters

The animals' fasting blood glucose was measured on days 0, 7, 14, 21, and 28. The blood sample was used to estimate glucose (ACCU-CHEK glucometer; Roche, Germany). The animals were anesthetized at the end of the administration period, and blood samples were collected via cardiac puncture. The blood samples were stored in clean vacutainer tubes and centrifuged at 4000 g for 15 minutes. The serum was used for the estimation of biochemical markers such as total serum cholesterol, serum triglyceride, and high-density lipoprotein (HDL) cholesterol, using Randox Diagnostic kits

Statistical Analysis.

One-way ANOVA was used for statistical testing significance between the groups, followed by Duncan's post-hoc test. Data were represented as means ± standard deviation (M ± SD). P values < 0.05 were considered significant. Statistical analyses were performed using the SPSS version 22.

RESULTS

Table 1: Effect of *Dialum guineense* (Icheku) extract on blood glucose levels on day 0, 7th, 14th, 21st and 28th

Groups	Treatment	Day 0	Day 7	Day 14	Day 21	Day 28
1	Normal Control (Non-diabetic rats)	79.5 ± 1.0	86.2 ± 1.5	84.1 ± 1.6	81.1 ± 0.6	77.1 ± 1.1
2	Negative control (Diabetic non-treated)	359.5 ± 1.6	335.5 ± 1.2	362.1 ± 0.6	370.3 ± 2.0	389.0 ± 1.5
3	Positive-control (diabetic treated with 0.1mg/kg Glibenclamide)	301.2 ± 2.2	250.5 ± 1.4*	239.3 ± 1.6*	210.0 ± 2.5*	194.3 ± 1.3*

4	Diabetic treated with 200mg/kg of extract	342.1 ± 1.5	229.5 ± 2.6*	198.0 ± 0.3*	169.5 ± 0.4*	153.2 ± 1.1*
5	Diabetic treated with 500mg/kg of extract	330.5 ± 1.0	239.4 ± 1.3*	200.2 ± 1.6*	169.1 ± 3.0*	129.3 ± 2.4*
6	Diabetic treated with 1000mg/kg of extract	319.2 ± 0.6	249.5 ± 1.4*	171.2 ± 2.3*	109.7 ± 1.2*	80.5 ± 1.3*

Values are mean ± SD: n=5. Values are statistically significant *p<0.05 compared to the negative control group.

The data for the blood glucose level presented in Table 1 showed that the animals in the diabetic control group experienced severe hyperglycemia compared to normal animals. The blood glucose level in the negative control group was 359.5 ± 1.6 mg/dl on Day 0 and 389.0 ± 1.5 mg/dl on Day 28. The standard drug glibenclamide was shown to reduce the blood glucose level significantly, whereas the *Dialum guineense* extract at 200 mg/kg, 500 mg/kg, and 1000 mg/kg significantly ($P < 0.05$) decreased the fasting blood serum glucose level in the diabetic rats on Day 7,14,21 and 28 as compared to the negative control group.

Table 2: Serum Lipid Profile in alloxan-induced rats after oral doses of ethanol extract of *Dialum guineense* (Icheku)

Group	Treatment	CHOL (mg/dL)	TRIG (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)
1	Normal Control (Non-diabetic rats)	89.27±2.41	62.02± 1.50	40.16±0.21	62.90±2.12
2	Negative control (Diabetic non-treated)	121.89± 0.72	108.39± 1.12	55.15 ± 0.03	90.32± 0.31
3	Positive-control (diabetic treated with 0.1mg/kg Glibenclamide)	98.17±4.38*	58.92 ±1.00*	48.52 ± 0.15	42.03±4.02*
4	Diabetic treated with 200mg/kg of extract	75.13±1.08*	52.20 ±2.08*	40.40±0.25*	50.31±2.25*

5	Diabetic treated with 500 mg/kg of extract	58.69±0.13*	50.05± 1.21*	36.14±0.33*	55.34±1.33*
6	Diabetic treated with 1000mg/kg of extract	50.04±3.40*	45.50 ±2.10*	42.21±0.21*	58.03±4.31*

Values are expressed as mean±SEM (n=6). *p<0.05 when compared with the negative control. CHOL: Cholesterol, TRIG; Trigycride, HDL; High-Density Lipoprotein, LDL; Low-Density Lipoprotein

Data for the lipid profile of the normal animals and the *Dialum guineense* - treated diabetic animals are presented in Table 2. The cholesterol level in the diabetic animal showed a non-significant elevation compared to the healthy control animal. The *Dialum guineense* – treated groups showed a significant ($p<0.05$) decrease in cholesterol level compared to the diabetic control animals. Glibenclamide also significantly ($p<0.05$) decreased cholesterol levels in diabetic animals. Our results also showed a non-significant elevation in the level of triacylglycerol in the diabetic animal compared to the healthy control animals. Treatment with *Dialum guineense* extract in all the administered doses significantly lowered triacylglycerol levels in the diabetic animal to levels comparable to that of the normal control animals. This was similar to the effect of glibenclamide, which also significantly lowered the triacylglycerol level ($p < 0.05$) in the diabetic animals. Induction of diabetes elevated the HDL levels of the animals; however, this was statistically insignificant compared to the control animals.

DISCUSSION

A rise in blood glucose levels is linked to diabetes mellitus. Following the induction of alloxan on the experimental animals, there was a significant rise in glucose levels across all groups in this research. This supports alloxan's cytotoxic properties, including damage and death of pancreatic islet cells in rats. *Dialum guineense* ethanol extract significantly lowered fasting blood glucose levels ($p<0.05$). According to our findings, the extracts of *Dialum guineense* at dosages of 200, 500, and 1000 mg/kg body weight lowered blood glucose more than glibenclamide, the typical medicine used to treat diabetes. This finding demonstrated the efficacy of *Dialum guineense* as a diabetic treatment. Knowing that glibenclamide has negative health consequences, it is recommended that *Dialum guineense* be used to prevent and control diabetes because it has less or no side effects than regular medications. The lower glucose levels in

Dialum guineense-treated rats might be higher plasma insulin concentrations due to pancreatic cell regeneration, leading to better blood glucose translocation in peripheral tissue. This result agrees with the finding of Ukpabi-Ugo [17], who reported that the presence of antioxidants in *Justicia carnea* may be the reason for the reduction of fasting blood glucose after treatment.

The most prevalent problems associated with diabetes mellitus have been proven to be changes in lipid metabolism, shown as hyperlipidemia. Changes in lipid profiles in diabetes patients are a risk factor for cardiovascular disease in studies. The alloxan-induced diabetic rats in this study had hypertriglyceridemia, lower HDL levels, hypercholesterolemia, and a modest increase in LDL levels compared to the control animals. This is in line with Alaebo *et al.* [13] research, which found that diabetic rats had higher blood TG, TC, and LDLc than control rats. The hypertriglyceridemia and hypercholesterolemia seen in this study might be due to hormone-sensitive lipase activation due to insulin shortage and insensitivity, increasing free fatty acid mobilization from peripheral reserves. *Dialum guineense* treatment of diabetic rats improved HDL levels while lowering triglyceride and cholesterol levels. The ethanol extract of *Dialum guineense* to alleviate lipid metabolism changes in diabetic animals might be attributed to the presence of a phenolic component in *Dialum guineense* that aids in lipid profile normalization.

Conclusion

The results of this study show that oral administration of *Dialum guineense* at all dosages lowers blood glucose levels and reduces the hyperlipidemia associated with diabetes. However, we propose further research to determine which biomolecules are responsible for this impact and how they work.

Ethical consideration

Throughout the experiment, all the rats were housed at 25^{o}C in clean metal cages under normal daylight humid conditions. The rats were freely fed pellets, given tap water, and made available throughout the experiment as approved by the departmental committee on animal use guidelines, Michael Okpara University of Agriculture, Umudike on handling experimental animals.

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