

Comparative Effect of Ethanol Extracts of *Lonchocarpus cyanescens* (Elu) and *Dialium guineense* (Icheku) Leaves on the body weight, Blood glucose Level and Lipid profile of streptozotocin-Induced diabetes in male Wistar Albino Rats

¹Amu, Pascal A., ²Nwaka, Andrew C. ³Olisah, Michael C., ²Nwaka, Chinyere S.

¹Department of Science Laboratory Technology, Institute of Management and Technology (IMT), Enugu, Enugu State, Nigeria

²Department of Biochemistry, Chukwuemeka Odumegwu Ojukwu University, Uli Campus, Anambra State, Nigeria

³Department of Medical Biochemistry, Chukwuemeka Odumegwu Ojukwu University, Uli Campus, Anambra State, Nigeria Email: ac.nwaka@coou.edu.ng

ABSTRACT

Comparative effect of ethanol extracts of *Lonchocarpus cyanescens* (Elu) and *Dialium guineense* (Icheku) leaves on the body weight, blood glucose level and lipid profile of streptozotocin-induced diabetes in male wistar albino rats were evaluated. The rats were randomly separated into seven (7) groups of five (5) rats each in seven (7) different aluminum cages as follows: A - Normal rats treated with normal saline daily served as (normal control), B - diabetic rats untreated (positive control), C - diabetic rats treated with 5mg/kg body weight of glibenclamide daily (standard drug), D -diabetic rats treated with 100mg/kg body weight of Icheku extracts daily , E - diabetic rats treated with 200mg/kg body weight of Icheku extracts daily , F - diabetic rats treated with 100mg/kg body weight of Elu extracts daily , G - diabetic rats treated with 200mg/kg body weight of Elu extracts daily. All the groups had free access to feed and drinking water *ad libitum*. The body weight of the rats were taken at weekly intervals with electronic weighing balance, at the end of 28 days, the rats were bled while serum obtained from the blood were used for blood glucose level determination and lipid profile assays using standard biochemical methods. The results of the serum glucose level in the treated diabetic rats of Elu and Icheku extracts significantly decreased ($P < 0.05$) when compared to diabetic control. The extracts showed more effective in reduction of glucose level when compared to standard drug, glibenclamide. The results revealed decrease in the body weight changes of the treated diabetic rats with the plant extracts when compared to control group. The results of the lipid profiles showed a dose dependent significant decrease ($P < 0.05$) of the extracts in cholesterol, LDL, and triacylglycerite on treated diabetic rats when compared to normal control group. Results of this study therefore, suggests that the plants extracts could be helpful in management of diabetes, prevention of atherosclerosis and body weight reduction.

Keywords: *Lonchocarpus cyanescens*, *Dialium guineense*, diabetes mellitus, body weight and blood glucose

INTRODUCTION

Several investigations into the chemical and biological activities of plants have yielded compounds with properties useful for the development of modern synthetic drugs for management of several diseases including diabetes. In fact, there has been increasing demand for the use of plant products with antidiabetic activity due to low cost, easy accessibility and lesser side effects. Plant like *Dialium guineense* and *Lonchocarpus cyanescens* have been reported to possess both medicinal and nutritional properties capable of anti-diabetic effect [1].

The main goal of diabetes treatment is establishing of normal levels of blood glucose and preventing or delaying its metabolic complications [2]. Herbs, due to their ease of access and fewer side effects,

have been the main cure for several diseases such as diabetes mellitus in ancient medicine [3].

Long before the use of insulin became common, indigenous remedies were used for the treatment of diabetes mellitus and hyperlipidemia. There has been an increasing demand from patients for the use of natural products with antidiabetic and anti-hyperlipidemic activity. This is largely because insulin cannot be used orally and insulin injections are associated with the risk of hypoglycemia and impairment of hepatic and other body functions. The undesirable side effects and contra-indications of synthetic drugs, and the fact that they are not suitable for use during pregnancy, have made scientists

look towards hypoglycemic agents of plant origin. Many herbs and plant products have been shown to have anti hyperglycemic and anti-hyperlipidemic action [4]. There are numerous traditional medicinal plants reported to have hypoglycemic properties such as *Allium sativum* (Garlic), *Azadirachta indica* (Neem), *Vincarosea* (Nayantara), *Trigonella foenum* (Fenugreek), *Momordica charantia* (Bitterground), *Ocimum gratissimum* (Tulsi). *Dialium guineense* also known as Black Velvet Tamarind is an indigenous tropical forest fruit tree of the family Leguminosae. *Dialium guineense* is an important non-timber multipurpose agro forestry crop with a high potential. The potentials of *Dialium guineense* as food supplement, in herbal medicine and as source of energy are well documented [5]. *Dialium guineense* is a lesser known tropical forest fruit with high consumption but given a less priority in terms of research, production, improvement, storage and hence not domesticated [6].

Lonchocarpus cyanescens is known as 'Elu' in Yoruba, 'anunu' in Ibo, 'talaki' in Hausa, 'suru' in Tiv and 'ebelun' in Edo languages of Nigeria. *Lonchocarpus cyanescens* also known as "ALU" is a deciduous scandent shrub. The plant has alternate leaves, flat fruits which are 1-5 seeded, oblong pod pointed at both ends. The aerial parts yield an indigo, which is a useful colourant for cloth dyeing in West Africa since ancient times. The plant is utilized in traditional medicine, bioactivity effects of *Lonchocarpus cyanescens* have been demonstrated in its anti-inflammatory, anti-arthritic, anti-diabetic and its relief on ulcer. It has some additional pharmacological properties including antiviral, antifungal, anti-protozoal, and antibacterial activities [7].

Diabetes is one of the oldest known diseases of man whose devastating effect is increasing by the day and severity almost at epidemic level. It is a disease of disordered metabolism of carbohydrate, protein and fat which is caused by the complete or relative insufficiency of insulin secretion

and /or insulin action. The number of people suffering from the disease worldwide is increasing at an alarming rate with a projected 366 million people likely to be diabetic by the year 2030 as against 191 million estimated in 2000 [8]. Developing countries are the most affected because of expensive and inadequate treatments, coupled with the side effect associated with these drugs. Thus the search for a new drug with low cost, more potential and without adverse effects becomes inevitable 2000.

The available therapies for diabetes include insulin and many oral hypoglycemic agents, such as biguanids and sulfonylurea. However, the use of oral drugs is limited due to adverse side effects including hematological and gastrointestinal reactions, hypoglycemic coma and disturbance of liver and kidney functions; in addition, they are also not suitable during pregnancy. So, with regard to the issue of socioeconomic burden of diabetes, discovery of more effective and without side effect therapies are necessary [9]. Since ancient times, good data have been obtained from traditional medicines indicating usefulness of many herbal medicines.

A great number of medicinal plants have been used in the treatment of diabetes in different parts of the world, some of which are without scientific scrutiny although World Health Organization (WHO) [10] had encouraged and recommended the use of plants as an alternative therapy for diabetes. Evaluation of the antidiabetic potentials of these plants becomes necessary to provide scientific proof and justify their uses in ethnomedicine [11]. There is claim by traditional herbalist on the effects of (Elu) and (Icheku) leaves on the treatment of diabetes and other associated diseases. Based on this, there is need to conduct a study in order to obtain scientific explanation on their effects on the body weight, blood glucose level and lipid profile using albino rats as model.

MATERIALS AND METHODS

Collection of Plant Materials

Lonchocarpus cyanescens (Elu) and *Dialium guineense* (Icheku) leaves used in this research work were freshly obtained from Lejja in Nsukka, L.G.A. Enugu State Nigeria and were botanically identified and authenticated by a botanist as *Lonchocarpus cyanescens* (Elu) and *Dialium guineense* (Icheku) at the Botany

Department, University of Nigeria, Nsukka before usage in this study.

Extraction of Plant Materials

The collected plant samples were rinsed in clean water and dried under ambient temperature for seven (7) days. The dried plant samples were milled into powder using electric blender, the powder obtained were then used to prepare the extract.

Preparation of Plant Extracts

The 100g of each of the powdered leaves were weighed with electrical weighing balance into sterilized conical flask and 500ml of distilled water was poured into the flask, the content of the flask was shaken and the top were covered with aluminum foil and kept at ambient temperature for 48 hours after which the extract were obtained by filtering using clean cloth with fine pores. The extracts were then concentrated in crucible using water bath set at temperature of 45°C. The weight of concentrated extract was taken and then stored in air-tight sample bottle in refrigerator till it is time to be analyzed.

Preparation of Streptozotocin

The streptozotocin was in a lyophilized form and therefore needed to be in an aqueous form for its administration to be possible. Therefore 7.5ml of citrated buffer was measured out and added to the powdered streptozotocin and was left for about 5minutes after which the mixture was gently shaken.

Induction of Experimental Diabetes in Rats

Diabetes was induced using 5mg/kg body weight of streptozotocin by intra-peritoneal injection to each of the fasting rats and acclimatization was allowed as described by [12]. After 72hours the blood glucose concentration was measured by one touch ultra-glucometer to confirm their blood glucose levels on the rats before the extracts and the standard drug was given. Diabetes was confirmed after 72 hours later in rats showing fasting blood sugar level \geq 200 mg/dl using an ultra-glucometer Analyzer.

The glucose level was checked every 7 days throughout the period of study.

Experimental Design

A total of thirty-five (35) male wistar albino rats weighing 150 - 200g were used for this study. The rats were obtained from the animal house in Faculty of Veterinary Medicine, University of Nigeria Nsukka, Enugu state Nigeria. The rats were housed at the animal garden Biochemistry Department University of Nigeria Nsukka. They were acclimatized for 10 days in steel cages. The rats were randomly separated into seven (7) groups of five (5) rats each in seven (7) different aluminum cages as follows: A - Normal rats treated with normal saline daily served as (normal control), B - diabetic rats untreated (positive control), C - diabetic rats treated

with 5mg/kg body weight of glibenclamide daily (standard drug), D -diabetic rats treated with 100mg/kg body weight of Icheku extracts daily, E - diabetic rats treated with 200mg/kg body weight of Icheku extracts daily, F - diabetic rats treated with 100mg/kg body weight of Elu extracts daily, G - diabetic rats treated with 200mg/kg body weight of Elu extracts daily. All the groups had free access to feed and drinking water *ad libitum*, while the rat feed used in the study was the Growers mash of vital*feed limited, Jos plateau state Nigeria. The body weights of the rats were taken at weekly intervals with electronic weighing balance, while the experiment lasted for 28 days. All the protocols as approved by Institutional Animal Ethics Committee (IAEC) were observed in this study.

Collection of Blood Sample from animals for Biochemical Analysis

Blood samples were collected from the animals through medial canthus of the eye of the rats for lipid profile analyses. The serum samples were put into a specimen plainbottles without anticoagulant. Serum sample were separated from the clot by centrifugation at 3000rpm for 5minutes using bench top centrifuge (MSE Minor, England). Serum samples were separated into plain tubes and stored in the refrigerator for analyses. All the analyses were completed within 24hours of sample collection.

Blood Glucose Level

The blood glucose of the animal was examined using AccuCheck glucometer machine with the blood samples obtained from the tail vein puncture of the animal. This method followed the standard procedures prescribed by the producer, Roche diagnostic company, Germany. The glucose level was checked every 7 days throughout the period of study

Biochemical Analysis of Serum Lipid Profile:

The serum lipid profile was assayed using Quimica Clinica Aplicada (QCA) test kits (QCA, Spain). The serum total cholesterol (TC) was determined by the enzymatic method,[13]. The serum high density lipoprotein cholesterol (HDL-C) was determined by the dextran sulphate-magnesium precipitation method [13]. The glycerol phosphate oxidase enzymatic method was used to determine the serum triacylglyceride [14] while the serum low density lipoprotein cholesterol (LDL-C) was calculated using the method of [15].

Statistical Analysis

The statistical analysis of the result was analyzed with statistical package for social science (SPSS) to obtain the mean and the standard deviation of the triplicate of result data for the descriptive analysis. Duncan

table was used to obtain the comparative result of the various groups of the study, while $P < 0.05$ (95 %) confidence interval was considered for the statically analysis.

RESULTS

Results in (Table 1) revealed that rats treated with the plants had a reduction in body weight when compared to normal control group. Also, there was a reduction in body

weight of the rats in diabetic control group across the groups. There was also reduction of body weight of the rats treated with standard drugs; glibenclamide.

Table 1: Body weight changes at Weekly Interval of Experimental Groups of Rats used in the study

Group	Baseline	1st Week	2nd week	3rd week	4th week
A	190.25 ± 5.22 ^a	195.25 ± 5.27 ^a	198.00±1.29 ^b	197.25±2.32 ^a	202.50±1.94 ^a
B	194.26±4.06	190±5.40	175.25±3.52	138.20±2.30	120.60±1.84
C	192.60 ± 3.70 ^a	140.80±35.29 ^b	153.00±5.81 ^a	127.00±6.00 ^b	165.00±2.00 ^b
D	182.80±40.04 ^a	173.00±4.44 ^{ab}	166.20±3.51 ^b	157.40±3.53 ^a	148.60±2.80 ^a
E	187.40 ± 3.85 ^a	160.75±3.52 ^{ab}	188.70±3.20 ^b	148.33±5.84 ^b	16220±3.06 ^b
F	191.20 ± 6.02 ^a	176.60 ± 4.80 ^a	164.00±2.81 ^b	155.20±2.24 ^a	130.40±0.93 ^a
G	186.80 ± 6.48 ^a	176.40±5.52 ^{ab}	163.20±5.59 ^a	149.0 ± 4.83 ^b	144.20±4.20 ^b

The values are expressed as the Mean ±SD of 6 determinations $P < 0.005$ compared with control

The results in (Table 2) showed a significant increase ($P > 0.05$) in blood glucose level of diabetic control rats when compared to normal rats. The oral administration of Elu and Icheku plant extracts showed a significant decrease ($P < 0.05$) in the blood glucose level of treated rats, compared to the control values of diabetic rats as well as compared to before treatment (day 0). There was also a significant decrease ($P < 0.05$) in the blood glucose level of rats treated with glibenclamide when compared to diabetic rats in control groups. The blood glucose levels of rats treated with Icheku 100mg/kg and 200mg/kg respectively showed the highest reduction on the 28th day (Table 2) of treatment when compared to Elu plant extract.

Table 2: Blood Sugar Levels in streptozotocin induced diabetic rats used in the study. After Treatment

Group	Day 0	7th Day	14th Day	21st Day	28th Day
A	119.25 ± 8.53 ^a	113.00 ± 5.67	115.25 ± 6.10 ^a	115.25 ± 9.80 ^a	78.25 ± 5.63 ^a
B	401.30±25.2 ^a	416.75±18.75	490.00±38.12 ^a	450.50±3.56	461.00±3.56
C	390.20 ± 2.01 ^a	398.40±99.56	326.20±136.69 ^{ac}	158.40±99.15 ^a	127.80±17.08 ^b
D	402.80±86.0 ^a	413.60±5.05	227.40±5.94 ^a	110.60±4.57 ^a	84.00±6.22 ^a
E	385.60±83.53 ^a	371.00±84.57	211.80±73.90 ^a	113.00±47.59 ^a	72.00±35.18 ^a
F	370.16±5.44 ^a	368.00 ± 7.50	213.20±3.72 ^{ab}	118.20± 3.68a	104.20±9.06 ^a
G	415.01±10.37 ^b	408.80±40.52	328.20±51.63 ^a	197.20±48.77 ^a	138.80±20.79 ^a

The values are expressed as the Mean ±SD of 6 determinations $P < 0.005$ compared with control

The results in (Table 3) revealed a significant decrease ($P < 0.05$) in Total Cholesterol, Low Density Lipoprotein and Triacylglyceride concentrations of rats treated with Elu and Icheku leaves extracts. However, there was a significant increase ($P < 0.05$) in High Density Lipoprotein-Cholesterol concentrations of rats treated with Elu and Icheku extracts when compared to normal control group rats. Also, the results obtained revealed a significant increase in Total Cholesterol, High Density Lipoprotein-Cholesterol, Low Density Lipoprotein-

Cholesterol and Triacylglyceride concentration of diabetic control rats when compared with treated rats and normal control rats

Table 3: Lipid Profile of Different Groups of Rats used in the Study.

Group	TC (mg/dL)	HDL-C(mg/dL)	LDL-C (mg/dL)	TG (mg/dL)
A	82.1±6.4 ^a	32.2±0.71 ^a	63.25±0.14	64.08±0.20 ^a
B	112.0±21.40 ^a	41.30±0.52 ^{bc}	95.34±0.18 ^{ab}	108.16±0.43
C	50.1±1.69 ^a	42.27±0.51 ^a	35.40±0.25	47.56±0.53 ^a
D	40.1±1.50 ^{ab}	38.41±0.41 ^{ab}	42.36±0.28 ^a	39.42±0.25 ^{ba}
E	35.2±0.47 ^a	40.35±0.63 ^a	51.34±0.25 ^a	40.36±0.90 ^{ab}
F	70.2±0.34 ^a	37.29±0.97 ^{ab}	60.32±0.3 ^{ab}	62.43±0.14 ^a
G	75.3±1.64 ^a	43.95±1.35 ^a	65.38±0.34 ^a	61.38±0.13 ^a

The values are expressed as the Mean ±SD of 6 determinations P<0.005 compared with control

Keys

A - This group was orally administered with normal saline and feed (Normal control).

B - This group was induced with 5mg/kg body weight of streptozotocin without being treated (Diabetic control).

C - This group was induced with 5mg/kg body weight of streptozotocin and treated with 5mg/kg body weight of glibenclamide.

D -This group was induced with 5mg/kg body weight of streptozotocin and treated with 100mg/kg body weight of the Icheku extract.

E - This group was induced with 5mg/kg body weight of streptozotocin and treated with 200mg/kg body weight of the Icheku extract.

F - This group was induced with 5mg/kg body weight of streptozotocin and treated with 100mg/kg body weight of the Elu extract.

G - This group was induced with 5mg/kg body weight of streptozotocin and treated with 200mg/kg body weight of the Elu extract.

DISCUSSION

The body weight changes at weekly intervals of the experimental animals were shown in Table 1. The results of this study in (Table 1) showed that streptozotocin-induced diabetic rats had significant decrease (P<0.05) in the body weight, when compared to the normal control group. This development is an evidence of disease in the body of the animals which was the major signs and symptoms of diabetes in the animals.

The study also observed that the oral administration of ethanol extracts of Elu and Icheku leaves at 100mg/kg and 200mg/kg respectively significantly decreased (P<0.05) the body weight of the rats when compared to diabetic control rats and normal control group. Moreover, the glibenclamide decreased the body weight of the rats when compared to diabetic control group and normal control group. However, the administration of ethanol extract of Elu on treated diabetic rats showed more significant decrease (P<0.05) in body weight after 28 days of treatment when compared to Icheku extract. The result of the studies suggests that Icheku and Elu extracts could be useful in stabilizing body weight.

The result of this study also showed that the negative control had a significant increase (P<0.05) in blood glucose level when compared to normal rats. From the result in Table 2 the administration of dose dependent of ethanol extracts of Elu and Icheku respectively showed a high significant reduction (P<0.05) in the serum blood glucose level starting at the 14th day of treatment when compared to diabetic control rats in the corresponding days as well as compared to before treatment (day 0). The study also observed that the plant extract of Icheku leaf had more reduction of serum blood glucose level across the treatment group when compared to the plant extract of Elu.

This could be an evidence that Icheku leaf extract contains more medicinal value and antioxidant property. The two plants extract had more effective reduction of blood glucose level in the 28th day of treatment which also brought the serum blood glucose to normal level when compared to the normal control group of the rats.

On the other hand, the positive control rats showed a significant reduction in blood glucose level across the treatment days

when compared to control diabetic rats in the corresponding days. The effect of these two plants extract in reduction of blood glucose level in diabetic rats is similar to that of glibenclamide (5mg/kg), a potent hypoglycemic drug. The significant reduction of the blood glucose level of the diabetic rats treated with the two plants extract in a dose dependent value could be attributed to the antihyperglycemic effect of the extracts which is in agreement with earlier studies on plants as alternative source of antihyperglycaemic drugs. This could be an evidence of its anti-hyperglycemic activity due to the presence of significant concentrations of bioactive components from the plants. [16] had a similar result on the effect of ethanolic root and twig extracts of utazi on streptozotocin induced hyperglycemic albino rats.

The results in Table 3 revealed that there was a significant ($P<0.05$) decrease in serum total cholesterol level of treated streptozotocin-induced diabetic rats across the groups when compared with normal control group. However, significant ($P<0.05$) increase was observed in the serum total cholesterol of diabetic control rats (untreated) when compared to other groups. The result also indicated a significant decrease ($P<0.05$) in serum level of LDL cholesterol and triacylglyceride (TG) concentration in all groups except diabetic groups when compared with normal control groups. However, significant increase ($P<0.05$) in serum LDL and TG concentration were observed in diabetic control groups (untreated).

The results of the lipid profile also revealed that there was a significant increase ($P<0.05$) in serum level of HDL-Cholesterol across all the groups when compared with normal control group.

The diabetic control rats had elevated mean total cholesterol, high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C) and triacylglyceride (TG) as observed in (Table 3). The results agreed with [17] who reported that high levels of triacylglycerides, LDL-C, have been associated with heart disease, insulin resistance and diabetes mellitus and that the increase in blood sugar was accompanied by marked increase in cholesterol, TG, LDL-C and reduction in HDL-C.

The results of the study observed that all doses of Elu and Icheku and glibenclamide significantly ($P<0.05$) reduced cholesterol, TG, LDL-C across the groups in the diabetic treated rats when compared with the normal

control group of rats used in the study. The study also revealed the significant increase ($P<0.05$) in serum level HDL-C of the ethanol extracts of Elu, Icheku and glibenclamide in the diabetic treated rats.

The reduction in both cholesterol, triacylglyceride and LDL-Cholesterol in treated diabetic groups suggests that the extracts of Elu and Icheku possibly produced effects due to the presence of phytoconstituents like tannin, saponin and other phytoconstituents which possibly decreased absorption of dietary cholesterol. Also, the observed decreased in serum cholesterol, LDL-C, triacylglyceride may be attributed to the bioactive phytoconstituent of the extracts like flavonoids and saponin which had been reported [18].

However, this study suggests a beneficial effect of the extracts in pathological conditions considering the phytochemical constituent of the plants. Therefore, the reduction in cholesterol level with the plants extracts is an indication that it could be used to reduce the incidence of coronary heart disease and atherosclerosis and other related diseases. The reduction in total cholesterol and LDL levels observed by the administration of ethanol extracts of the plants demonstrated a possible protection against hypercholesterolemia.

HDL Cholesterol is known to have a protective effect against cardiovascular disease since it can remove excess cholesterol from circulation and carries it back to the liver where it is degraded into bile acid. Also HDL is an anti-atherogenic lipoprotein which transports cholesterol from peripheral tissues into the liver thereby could act as a protective factor against coronary heart disease.

The present study observed that the significant increase ($P<0.05$) in serum level HDL-cholesterol suggested that the ethanol leaf extract of the two plants and the glibenclamide may be used to reduce the risk factor of atherosclerosis and other cardiovascular related disorders.

Ethanol extracts of Elu and Icheku therefore have shown hypolipidemic effects in diabetic rats.

LDL-Cholesterol plays an important role in arteriosclerosis and hypercholesterolemia. However, the observed significant reduction ($P<0.05$) in low density lipoprotein (LDL-cholesterol) following the administration of the extracts on diabetic treated rats suggests that the extracts possess cholesterol lowering ability which might be a great benefit in the management of

atherosclerosis and other cardiovascular related disorders. The decrease in LDL cholesterol of the plants extracts suggest a possible protection against hypercholesterolemia.

The triacylglyceride level of the rats treated with the plants extracts and glibenclamide in streptozotocin- induced diabetic rats decreased significantly ($P < 0.05$) when compared with normal control group and diabetic control group (untreated). Under normal conditions, insulin activates the enzyme lipoprotein lipase which hydrolysis triacylglycerides. Also in the diabetic state,

From the results of the study it was observed that there was a decrease in the body weight of the rats treated with the extracts of Elu and Icheke which may suggest that it could be good supplement for body weight reduction.

The results of the study also revealed a decrease ($P < 0.05$) in total cholesterol, LDL-chol. and TAG concentrations of rats treated with the extracts when compared to the control. Since the level of plasma lipids are elevated in cardiovascular diseases, this study suggests that Elu and Icheke extracts

lipoprotein is not activated due to insulin deficiency resulting in hypertriacylglyceride. The reduction in triacylglyceride by the plants' extracts could be a good factor in reducing the liver disease. The results obtained in the lipid profile analysis showed that Icheke plant extract brought about more effective reduction in TC, HDL, LDL-chol. and TG when compared to Elu plant extract as shown in Table 3. Therefore, the study suggests that Icheke plant extract could be more useful in normalizing lipid profile of the animal than Elu extract.

CONCLUSION

could be useful in prevention and management of cardiovascular diseases. Also this study revealed the hypoglycaemic and hypolipidemic property of Elu and Icheke and suggests that the extracts are safe and potent agent capable of bringing to normal, glucose level and lipid abnormalities associated with diabetes mellitus and as such could be used in the management of the disease. The plants extracts could also be used in the management of diabetes mellitus, since it decreased the blood glucose of the rat

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