EVALUTION OF ANTI-DIABETIC POTENTIAL OF AQUEOUS EXTRACT OF "*LUFFA CYLINDRICA*" (NATIVE SPONGE/SPONGE GOURD) LEAF AND SEED ON ALLOXAN INDUCED DIABETIC WISTAR RATS.

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ABSTRACT

The study was carried out to evaluate the anti-diabetic effect of *Luffa cylindrical* (native sponge /sponge gourd) seed and leaf extracts in alloxan- induced diabetic rats. Sixteen experimental rats were divided into four groups of four rats each: a, diabetic control; b, normal control; c, diabetic rats treated with seed extract (400mg/kg) and d, diabetic rats treated with leaf extract (400mg/kg). The groups A, C and D rats were induced with diabetes intraperitoneally with alloxan (150mg/kg bw). Phytochemical screening was carried out on the plant seed and leaf extracts and the following biochemical tests were carried out: blood glucose, serum lipid profile, serum alanine aminotransferase, serum aspartate aminotransferase, serum alkaline phosphatase, total protein, albumin, creatinine, urea, uric acid and some electrolytes like Na^+, K^+, HCO_3^-, and Cl^- the administration of alloxan to experimental rats resulted in an increased level of most biochemical parameters; blood glucose, serum alanine aminotransferase, serum aspartate aminotransferase and serum alkaline phosphatase, serum total cholesterol, triglyceride, low density lipoprotein, creatinine, urea and uric acid. *Luffa cylindrica* seed and leaf extracts was administered to groups c and d diabetic rats respectively for two weeks, results were compared with normal control and diabetic control rats these parameters were found to be significantly ($p<0.05$) high in the diabetic groups than in the normal control groups. Treatment with the plant extract significantly ($p<0.05$) reduced elevated blood levels of glucose, cholesterol, triglyceride, alkaline phosphatase, amylase, aspartate aminotransferase, alanine aminotransferase, creatinine, urea, uric acid associated with alloxan-induced diabetic rats. The plant tested positive for alkaloids, flavonoids, saponins and tannins, negative for cardiac glycosides, phenols, resins, terpenes and steroids. Extracts of *Luffa cylindrica* seed and leaf has shown to have anti-diabetic and anti-lipidemic effects generally on alloxan induced diabetic rats. The study's findings has shown that the plant possess hypoglycaemic and hypolipidaemic property and has supported the traditional use of *Luffa cylindrica* plant in the management of diabetes and its complications.

Keywords: Luffa cylindrical, alloxan, leaf, seed, diabetes

1. INTRODUCTION

A number of plants have been used in traditional medicine for many years. Some do seem to work although there may not be sufficient scientific data (double-blind trials, for example) to confirm their efficacy. Such plants should qualify as medicinal plants. The term 'crude drugs of natural or biological origin' is used by pharmacists and pharmacologists to describe whole plants or parts of plants which have medicinal properties [1]. In view of the fact that at the time there was not sufficient information either concerning the reasons for the illnesses or concerning which plant and how it could be utilized as a cure, everything was based on experience. In time, the reasons for the usage of specific medicinal plants for treatment of certain diseases were being discovered; thus, the medicinal plants' usage gradually abandoned the empiric framework and became founded on explicatory facts. Until the advent of iatrochemistry, plants had been the source of treatment and prophylaxis [2]. Nonetheless, the decreasing usefulness of synthetic drugs and the increasing contraindications of their usage make the usage of natural drugs topical again. Traditional medicine has been used by the majority of the world population for thousands of year [3].

Fig. 1: pictorial representation of the *Luffa cylindrica* plant

Amongst all the medicinal plants used in Nigeria for management and treatment of various types of aliments is the native sponge, scientific name is *Luffa cylindrica* in figure 1 above. It has other common names as smooth Luffa, sponge Luffa, vegetable sponge gourd, climbing okra, dishcloth gourd, chinese okra, it belongs to the family cucurbitaceae. *Luffa cylindrica* is native to India. Locations within which *Luffa cylindrica* is naturalized include: eastern africa and some pacific islands. *Luffa cylindrica* is naturalized in parts of Nigeria, Kenya and Tanzania and invasive in parts of Uganda.

Luffa cylindrica (sponge gourd) belonging to family *cucurbitaceae* is widely used across the globe as a vegetable. *L. Cylindrica* roem fruit extract (lce) has been found to be an excellent antidiabetic and antioxidant[4][5].

Luffa cylindrica as a medicinal plant has been widely active in treatment of many diseases and used in proffering solutions to clinical problems relating to child birth. Although too many communities where this plant is used have little idea about the secret of its potency. Scientific research has shown the presence of some chemical components and proteins in *Luffa cylindrica*, and many others, which made it possible for *Luffa cylindrica* to be used as potentially effective chemical agent in health care delivery. Thus, possibility of transforming the chemical agents implicated in the plant of study into synthetic drugs to combat endemic diseases such as cancer and HIV should be the next focus of the clinical scientists [1].

Diabetes is a disease in which the body's ability to produce or respond to the hormone insulin is impaired, resulting in abnormal metabolism of carbohydrates and elevated levels of glucose in the blood. Diabetes mellitus is a clinically and genetically heterogeneous group of disorders that has a common feature of abnormally high levels of glucose in the blood due either to insulin deficiency or to resistance of the body's cells to the action of insulin. Diabetes mellitus or commonly diabetes is considered to be one of most serious, endocrine syndrome. It is a metabolic disorder characterized by hyperglycemia, glycosuria, hyperlipidemia, negative nitrogen balance, and sometimes ketonemia. Type 1 diabetes is caused by deficiency of insulin secretion from β-pancreatic cells [5]. On the other hand, type 2 diabetes is characterized by initial phases of progressive insulin resistance.

2. MATERIAL AND METHODS

Plant material

Luffa cylindrica leaves were obtained from a growing tendril *Luffa cylindrica* plant, from kafachang kaduna state of Nigeria while the seeds were also obtained from a growing tendril *Luffa cylindrica* plant, From Jos, Plateau State of Nigeria and they were both identified in the Biochemistry Laboratory Of Bingham University, Karu Nassarawa State Nigeria.

Preparation and Administration of The *Luffa Cylindrica* Leaves and Seeds Extract

Luffa cylindrica leaves and seed extract was prepared by drying of the leaves and seeds collected. The *Luffa cylindrica* leaves and seed were pounded and matched to powder, 100g

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and 50g of the samples were weighed respectively and was soaked in 1000ml and 500ml of distilled water (100mg/ml and 50mg/ml respectively) respectively and then stirred and heated on a hot plate for 15mins. The extracts were filtered with cloth sieve and then heated in the water bath to dry at 60℃ till samples completely dry. 400mg of *Luffa cylindrica* plant extracts per kg body weight of rats was administered to each rat of each group of extract once a day.

Experimental Specimen

Albino rats of 150-200g weight were purchased from Plateau State Nigeria. The experiment was approved by the HOD Biochemistry Bingham University Karu, Nassarawa State and HOD Animal Farm Unit of University Of Jos, Plateau State, Nigeria. The rats were housed in metal cages with steel net covers and kept at room temperature (24-28℃) under 12hours dark-light cycles. All rats were fed appropriately with their respective diet feed, water and were acclimatized for 2 weeks in the animal house in University Of Jos, Plateau State.

Induction of Diabetes

Alloxan was induced in experimental rats after 12hours of fasting (overnight) by intraperitoneally administration of 150mg/kg body weight of alloxan. After the above observations, the fasting blood glucose concentration of all experimental rat were determined with the aid of a glucometer (blood was taken from the respective rat's tail) for concentration greater than 120mg/dl.

Experimental Design

The control and experimental rats divided into different groups and treated accordingly;	
Group 1:	normal control: non-diabetic group
Group 2:	diabetic control: diabetic group
Group 3:	*Luffa cylindrica* leaves extract group: diabetic rats receiving 400mg *Luffa cylindrica* leaves extract per kg body weight once daily.
Group 4:	*Luffa cylindrica* seeds extract group: diabetic rats receiving 400mg *Luffa cylindrica* seeds extract per kg body weight once daily.

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Determination Of Biochemical Parameters

Determination of uric acid

Determination of blood glucose

The fasting blood glucose concentration was determined by o'toluidine method.

O'toluidine solution

Determination of serum total protein

Biuret reagent

Determination of serum total cholesterol

3. RESULT AN DISCUSSION

Statistical analysis

The data were expressed as Mean ± standard error of Mean. Statistical analysis was performed using analysis of variance (anova) at 5% level of confidence ($p<0.05$). Using spss analytical software.

Results

Table 1: percentage extraction of plant samples extracted and used on the experimental rats.

Sample	Weight of raw plant (g)	Weight of plant extract (g)	Percentage of plant extraction (%)
Leaves	100	13.35	13.35
Seeds	50	7.60	15.2

Table 2: phytochemicals present in the plant samples (leaves and seeds) used in the analysis and administered to experimental rats.

S/n	Phytochemicals	Leaf	Seed

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1.	Alkaloids	+	+
2.	Flavonoids	+	+
3.	Tannins	+	-
4.	Saponins	+	+
5.	Terpenes	-	-
6.	Steroids	-	-
7.	Cardiac glycosides	-	-
8.	Balsam	+	-
9.	Carbohydrates	+	-
10.	Phenol	+	-
11.	Resins	-	-

111 Key

112 + = detected - = not detected

113 Table 3: effect of administration of *Luffa cylindrica* seeds and leaves extract on biochemical
114 parameters (such as glucose level, total protein and albumin).

Group	Treatment	Glucose (mmol/l)	Total protein (g/l)	Albumin (g/l)
A	Diabetic control	12.46±0.022	68.50±0.426	30.42±0.510
B	Normal control	3.48±0.029[a]	76.47±0.442[a]	38.50±0.430[a]
C	Diabetic + seed	8.34±0.029[ab]	70.47±0.521[ab]	33.44±0.464[ab]
D	Diabetic + leaf	6.70±0.037[ab]	73.39±0.447[ab]	31.58±0.457[ab]

115 Values are expressed as Mean ± SD, n= 4 for each group.
116 [A]values are significantly different when compared with diabetic control ($p<0.05$).
117 [B]values are significantly different when compared with normal control ($p<0$).

118

119 Table 4. Effect of administration of *Luffa cylindrica* seeds and leaves extract on lipid profile
120 parameters.

Group	Treatment	Total cholesterol (mmol/l)	Triglyceride (tg) (mmol/l)	High density lipoprotein (hdl) (mmol/l)	Low density lipoprotein (ldl) (mmol/l)
A	Diabetic control	5.35±0.103	1.98±0.166	0.52±0.089	2.26±0.317
B	Normal control	3.14±0.025[a]	0.80±0.138[a]	1.31± 0.257[a]	1.44±0.178[a]
C	Diabetic + seed	4.45±0.029[ab]	1.64±0.173[ab]	0.78±0.129[ab]	1.88±0.245[ab]
D	Diabetic + leaf	3.46±0.033[ab]	1.21±0.166[a]	0.88±0.141[ab]	1.63±0.171[ab]

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122 Values are expressed as Mean ± SD, n= 4 for each group.
123 [A]values are significantly different when compared with diabetic control ($p<0.05$).
124 [B]values are significantly different when compared with normal control ($p<0.05$).

125 Table 5: effect of administration of *Luffa cylindrica* seeds and leaves extract on some liver
126 function test parameters {ALT (Alanine Aminotransferase), AST (Aspartate
127 Aminotransferase), and ALP (Alkaline Phosphatase)}

Group	Treatment	ALT (u/l)	AST (u/l)	ALP (u/l)
A	Diabetic control	24.53±0.830	31.49±0.843	359.35±0.520
B	Normal control	12.58±0.822[a]	16.50±0.684[a]	168.41±0.724[a]
C	Diabetic + seed	18.74±0.827[ab]	23.52±0.874[ab]	284.31±0.602[ab]
D	Diabetic + leaf	15.76±0.708[ab]	19.59±0.852[ab]	251.55±0.731[ab]

128 Values are expressed as Mean ± SD, n= 4 for each group.

129 [A]values are significantly different when compared with diabetic control ($p<0.05$).
130 [B]values are significantly different when compared with normal control ($p<0.05$).

131 Table 6: effect of administration of *Luffa cylindrica* seeds and leaves extract on other
132 biochemical parameters (such as creatinine, urea and uric acid).

Group	Treatment	Creatinine (µmol/l)	Urea (mmol/l)	Uric acid (µmol/l)
A	Diabetic control	11.54±0.420	197.53±0.501	408.30±0.580
B	Normal control	4.34±0.477[a]	104.48±0.437[a]	268.30±0.658[a]
C	Diabetic + seed	9.44±0.435[ab]	159.70±0.480[ab]	314.25±0.645[ab]
D	Diabetic + leaf	7.35±0.505[ab]	124.44±0.433[ab]	351.55±0.701[ab]

Values are expressed as Mean ± SD, n= 4 for each group.

[A]values are significantly different when compared with normal control ($p<0.05$).

[B]values are significantly different when compared with diabetic control ($p<0.05$).

Table 7 Effect of administration of *Luffa Cylindrica* seeds and leaves extract on biochemical electrolytes parameter (Such as Sodium, Potassium, Chloride and Biocarbonate

Group	Treatment	**Sodium** Na^+ (mmol/L)	**Potassium** K^+ (mmol/L)	**Chloride** Cl^- (mmol/L)	**Bicarbonate** HCO_3^- (mmol/L)
A	Diabetic Control	135.29±0.766	5.90±0.026	106.26±0.501	18.26±0.522
B	Normal control	141.29±1.056[a]	3.70±0.022[a]	113.28±0.684[a]	26.25±0.510[a]
C	Diabetic + Seed	137.00±0.816[ab]	5.20±0.026[ab]	108.28±0.643[ab]	21.25±0.506[ab]
D	Diabetic + Leaf	139.01±0.816[ab]	4.30±0.050[ab]	110.28±0.597[ab]	24.25±0.507[ab]

DISCUSSION

Treatment with *Luffa cylindrica* plant extracts (seed and leaf) produced a time dependent decreased concentration in blood glucose level and other biochemical parameters: total protein, total cholesterol and liver enzymes (alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase).

The high level of glucose observed in blood of induced experimental rats by the administration of alloxan, in our case, which is cytotoxic (toxic to living cells) specifically for the β-cells of the islets of langerhans in the pancreas which function in regulation of insulin secretion [6]

It has been identified that the liver is necrotized in alloxan induced diabetic rats which leads to release or increase activities of liver enzymes (alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase) as they leak due to cirrhosis from the liver to the bloodstream and this is an indicator of the hepatotoxicity caused by the induction of alloxan shown clearly in the diabetic group [7]. The time dependent decrease of these liver enzymes in the blood stream maybe due to the administration of the plant extracts which may have helped in retrogressing the hepatocellular damage caused by alloxan administration initially, thereby helping in refurbishing and mending the hepatocyte membrane integrity.

The table 3 above shows the result of the analysis of biochemical parameters (glucose, total protein and albumin) on experimental rats. In consideration of groups of the experimental rats, the glucose concentration of the normal control had no significant change in the concentrations, considering the diabetic + seed and diabetic + leaf treatment group, which was shown to be significantly different when compared respectively to the diabetic control group ($p<0.05$). As compared generally, the results shows that administration of *Luffa cylindrica* plant extracts (seed and leaf) were effective in reducing blood glucose level after 14days of treatment, as earlier observed in the same research carried out utilizing *Luffa cylindrica fruits* which tested for antihyperglycemic activity in alloxan induced hyperglycemic rats [8].

Secondly, the total protein and albumin tests may be ordered in a variety of settings to help diagnose disease, to monitor changes in health status, total protein measurements can reflect nutritional status and may be used to screen for and help diagnose kidney disease or liver disease. A low total protein such as the diabetic group level can suggest a liver disorder, kidney disorder, or a disorder in which protein is not digested or absorbed properly. Low levels may be seen in severe malnutrition and with conditions that cause mal-absorption, such as celiac disease or inflammatory bowel disease [9]. The destruction of the pancreas results in the utilization of non-carbohydrate precursors such as protein for the synthesis of glucose to form energy need in the cells, generally leads to increased lipolysis and increased synthesis of ketone bodies results in severe decrease in the total protein level

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observed in diabetic group. The table 3 in the results above shows the result of the analysis of biochemical parameters (total protein and albumin) on experimental rats. After 14days of treatment and induction of diabetes in experimental rats it was observed that the concentration of the total protein comparing the diabetic control group of concentration which was shown to increase. Compared with that of the diabetic + seed and diabetic + leaf groups, which shows that all groups maintained the normal range of total protein which is between 60g/l to 80g/l. However, results indicate the ability of *Luffa cylindrica* plant extracts to be effective in enhancing the level of total protein observed in the extraction groups of seed and leaf when compared with the diabetic group, as also seen in the table ($p<0.05$). Also, after 14days of treatment and induction of diabetes in experimental rats it was observed that the concentration of serum albumin which measures the amount of liver protein contained in the clear liquid protein of the blood, when compared with the normal control group seemed to be significantly different from the diabetic + seed and diabetic + leaf groups ($p<0.05$). Also, in the same research, venous blood samples was used for estimation of plasma glucose, total proteins, albumin, fibrinogen which tested in the study of type 2 diabetics, plasma albumin levels were decreased compared to controls and plasma fibrinogen, total protein levels were statistically significantly increased compared to controls [10].

The increase in total cholesterol level of the diabetic group was due to the hyperglycemia confirmed in the diabetic group. *Luffa cylindrica* plant extracts were able to improve lipid metabolites generally including the correction of the high density lipoproteins known as good cholesterol which aids as carriers for the removal of low density lipoproteins and triglyceride from the blood to prevent the blockage of arteries, results indicates the ability of *Luffa cylindrica* plant extracts to be effective in correction of these levels of metabolites in experimental rats diabetic and treated respectively.

The table 4 in the results above shows the result of the analysis of biochemical lipid profile parameters (total cholesterol, triglyceride, high density lipoproteins and low density lipoproteins) on experimental rats. In consideration of the groups of the experimental rats (experimental design) the result of the serum total cholesterol, shows that *Luffa cylindrica* seed and leaf extracts is effective in significantly reducing the level of serum total cholesterol for diabetic control group ($p<0.05$). Comparing the diabetic + seed and diabetic + leaf extract groups, the leaf of the plant reflects the ability to reduce serum total cholesterol more than the seed of the *Luffa cylindrica* plant.

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After 14days of treatment and induction of diabetes in experimental rats it was observed that the concentration of the triglyceride and low density lipoproteins when compared with the diabetic control groups, which showed a significant decrease ($p<0.05$) on treatment with seed in the diabetic + seed group, which reflects that the *Luffa cylindrica* plant is effective in reducing the concentration of triglyceride and low density lipoproteins. Also, the result of the high density lipoproteins concentration after 14days of treatment with the *Luffa cylindrica* plant extracts, in the comparison with the diabetic group also shows that there is an increase in the level of the concentration of the high density lipoproteins in the diabetic + seed and diabetic + leaf groups respectively ($p<0.05$).

The table 5 in the results above shows the result of the analysis of some biochemical liver function test parameters (alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase) on experimental rats. Considering the groups of experimental rats it was observed that the concentration of the liver enzyme (serum ALT, AST and ALP) in normal control group increased significantly when compared to that of the diabetic control group ($p<0.05$).

After 14days of the administration of the seed and leaf extracts, there was a decrease in the diabetic + seed treatment group respectively of each of the enzymes and the diabetic + leaf treatment group also ($p<0.05$). The result shows that when comparing the diabetic + seed with diabetic + leaf the leaf treatment is more effective in reducing liver damage. But generally *Luffa cylindrica* plant extracts were effective in reduction of liver damage which result in high level serum alanine aminotransferase, serum aspartate aminotransferase and serum alkaline phosphatase.

The table 6 in the results above shows the result of creatinine, urea and uric acid on experimental rats, it was observed that the concentration of the creatinine, urea and uric acid in diabetic control group increased significantly when compared to the normal control group ($p<0.05$).

After 14days of the administration of the seed and leaf extracts, there was a decrease in the diabetic + seed treatment group and the diabetic + leaf treatment group ($p<0.05$). The result shows that when comparing the diabetic + seed with diabetic + leaf the leaf treatment was more effective and generally shows that *Luffa cylindrica* plant extracts were effective in reducing creatinine, urea and uric acid levels in the blood which aids in the reduction of kidney disease and dysfunction [12].

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Table 7 shows the result of the analysis of sodium, potassium, chloride and bicarbonate on experimental rats. They are generally chemicals in the blood stream that regulate important functions in the body, when dissolved in water electrolytes separates into positively and negatively charged ions, it was observed that the concentration of all the electrolytes except potassium in normal control group reduced significantly when compared with that of the diabetic control group ($p<0.05$) but that of potassium rather increased for normal and diabetic respectively. The result shows that when the diabetic + seed and diabetic + leaf treatments were both effective in reducing electrolyte imbalance which may have occurred due to hormonal or endocrine disorders, kidney disease and dysfunction.

Also, methanolic extract of *Luffa cylindrica* fruits on oral glucose tolerance and its effect on normoglycemic rats were studied [8]. The same was tested for antihyperglycemic activity in alloxan induced hyperglycemic rats at the two dose levels 200 and 400mg/kg body weight. The serum biochemical parameters were also assessed in the alloxan induced experimental animals. The methanolic extract of *Luffa cylindrica* exhibited remarkable antihyperglycemic activity [8]. The treatment of diabetic rats with methanolic extract of the test plant improved the serum biochemical parameters and the activities were found to be dose dependent, the respective effects were basically observed on fasted normal, alloxanised hyperglycemic and glucose tolerance rats. Diabetes is associated with profound alterations in lipid and lipoproteins profiles, early detection and treatment of hyperlipidemia in diabetic patients reduces the risk for cardiovascular and cerebrovascular diseases [13]. Therefore lowering of plasma or tissue lipids levels generally may lead to decrease in the risk of micro and macro vascular disease related complications [14]. It therefore can be suggested that *Luffa cylindrica* plant extracts may improve lipid profiles as shown in the results above either directly or indirectly through reduction of blood glucose level generally in experimental rats diabetic or treated respectively as observed in this research study.

4. **CONCLUSION**

In conclusion, *Luffa cylindrica* seed and leaf extracts were able to reduce elevated level of blood glucose, lipid profile and serum enzymes. The result confirms antidiabetic potential of *Luffa cylindrica* plant in alloxan induced diabetic wistar rats. The results suggest that *Luffa cylindrica* plant extracts' have the possibilities to improve and enhance treatment of diabetes complications.

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