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Protective Effects of *Luffa aegyptica* Aqueous Extract Against Biochemical Alterations in Diabetic Rats

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Abstract
Diabetes, a disease linked to intermediary metabolism is caused by reduced production of insulin or increasing resistance. The objective of this study was to assess the protective effect of Luffa aegyptiaca aqueous leaf extract (LAAE) against alterations in haematological indices, lipid profile, atherogenic index and hypoamylasemia in diabetic male rats. Thirty male Wistar rats were grouped into six of five animals each as follow: Normal control (NC), Diabetic Control (DC), E1 (diabetic rats + 100 mg/kg of LAAE), E2 (diabetic rats + 200 mg/kg of LAAE), E3 (diabetic rats + 300 mg/kg of LAAE) and STD (diabetic rats + metformin drug (100mg/kg)). The induction of diabetes using alloxan monohydrate solution (150 mg/kg), caused hyperlipidaemia, increased atherogenic and cardiovascular risk indices and hypoamylasemia in the rats. The extract administration increased the amount of high density-lipoprotein (HDL), triglyceride (TG) and total cholesterol. The extract reduced low density-lipoprotein (LDL), atherogenic index (AI) and coronary risk index (CRI). The levels of haematological indices [packed cell volume (PCV), red blood cell (RBC) and haemoglobin concentration (Hb)] increased upon the administration of the extract. Total white blood cells (TWBC), mean value of corpuscular haemoglobin (MCH), mean value of corpuscular haemoglobin concentration (MCHC), and mean value of corpuscular volume (MCV) reduced slightly in the treated animals when compared with the diabetic control ($P<0.05$). Liver integrity was restored by increase in the levels of serum total protein (STP), albumin and amylase activity. It was concluded that aqueous extracts of L. aegyptiaca possess therapeutic and protective effect on the liver, adipocyte and haematological indices of diabetic rats.

Keywords: *Luffa aegyptica*, diabetes, hyperlipidemia, atherogenic index, hypoamylasemia

Introduction
Diabetes is a disorder of intermediary metabolism ascribed to reduced production of insulin or increasing resistance [1]. Use of traditional herbs for the monitoring of diabetic patients has been in practice in Nigeria [2]. Scientific confirmation of several plant species has demonstrated the efficacy of the botanicals in reducing the sugar level [3]. There are numerous plants known for their antidiabetic property, with different mode of action and phytochemical constituents [4]. There is an effort to simplify the phytochemical constituents of specific family with specific mode of action to reduce plasma glucose [1,2]. *Luffa aegyptiaca*(Sponge gourd) has the most diverse uses of any of the cultivated cucurbits [5]. Immature fruits of the non-bitter genotypes are eaten fresh, cooked, or in soups, and is adapted for the treatment of varied diseases [6]. The quality controlled studies on fruit of *Luffa aegyptiaca* shows that aqueous extracts has maximum extractive power in comparison to other solvents such as hexane, ethanol, chloroform, ethyl acetate [3]. The *Luffa aegyptiaca* extracts when examined for the following test for purity e.g. acid insoluble ash, loss on drying, water soluble and sulphated ash shows that sample is rich in drug having large polar compounds [6,1]. Simple pilot analysis reveals that these extracts contain carbohydrates, reducing sugars, alkaloids, saponins, steroids, glycosides, compounds of phenol, flavonoids, quinines, tannins and lignins [5,6]. Flavonoids and tannins have been implored in the curing of cancers and heart related problems through food supplementation [6,7].

Materials and Methods
Experimental Animals
Adult male rats of three to four months weighing between 100-200g were gotten from the animal house, Faculty of Basic Medical Sciences, Delta State University, Abraka, Nigeria. They were fed with grower marsh obtained from Dutch Farm Limited, Abraka and water *ad libitum*. They were made to adapt to the environment for one week thereafter, induced with alloxan monohydrate, which causes hyperglycemia. The animals were handled following standard International Ethics on use of animals for experimental purposes.
Chemicals
Alloxan monohydrate and all other reagents used for this study were obtained from Alpha Chimika, Mumbia, China.
Collection and Identification of Plant Material

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The plants were collected from Warri, Delta State and identified at the Department of Botany, Delta State University, Abraka.

Preparation of Extract

Fresh leaves of *Luffa aegyptiaca* were washed to remove contaminants and air-dried for about three weeks. Thereafter, they were grinded to fine powder using Waren blender. 800g of powdered solute (*Luffa aegyptiaca*) was soaked in 3200 ml (3.2L) of distilled water in a ratio 1:4. It was macerated for 48hrs to obtain the crude extract. This was followed by filtration using Whatman No110 filter paper. The purified crude was then concentrated using a vacuum rotary evaporator at reduced temperature (50^{o}C) and thereafter evaporated further at 40^{o}C in a water bathe. This yielded a dark brown concentrated extract of 141.88 g (17.7%w/w). The obtained crude extract was packaged in an airtight plastic container and stored at 4^{o}C for analysis.

Induction of Diabetes

The rats were denied feeding for 12 hours and induced intraperitoneally (i.p) at a dosage of 150mg/kg alloxan monohydrate solution. Prior to their induction, the fasting blood glucose level (FBGL) was determined using glucometer to ascertain the glucose levels. The alloxan monohydrate solution was prepared by dissolving 4g of alloxan in 100ml of normal saline. This corresponds to a stock solution of 40mg/ml. After 72 hours of induction, hyperglycemic conditions of ≥200mg/dl were confirmed using a glucometer [8,9].

Experimental design

Thirty (30) rats, male by gender, were grouped into six with five rats each as follows:

NC: Negative control (Healthy rats+ no treatment)
DC: Diabetic control (Alloxan-treated rats + no treatment)
STD: Diabetic rats + Metformin (100mg/kg)
E1: Diabetic rats + 100 mg/kg of extract of L. aegyptica
E2: Diabetic rats + 200 mg/kg of extract of L. aegyptica
E3: Diabetic rats + 300 mg/kg of extract of L. aegyptica

Their blood glucose was monitored every four days for fourteen days.

Collection of Sample

The rats were sacrificed by cervical decapitation and blood sample was collected using 5ml syringe from each rat. Samples were emptied into anticoagulant containers to prevent clotting (plasma enzymes). The tubes were spanned at 3000rpm for 10mins. The supernatants was analysed for the following biochemical parameters.

Determination of Biochemical Parameters

The determination of lipid profile [total cholesterol (TC), triglcerides (TG), high density-lipoprotein- cholesterol (HDL) and low density-lipoprotein- cholesterol (LDL)], haematological indices [packed cell volume (PCV), haemoglobin concentration (Hb), red blood cell (RBC), total white blood cells (TWBC), mean value of corpuscular haemoglobin (MCH), mean value of corpuscular haemoglobin concentration (MCHC), and mean value of corpuscular volume (MCV)] and liver biomarkers [Total protein (TP), serum albumin (ALB) and serum amylase were carried out using the PrietestEasylab Biochemistry analyser, Lab Tech, England.

Statistical Analysis

The data was analysed using a computer software version 21 (SPSS 21). One-way analysis of variance (ANOVA) was used to compare the results. The results are expressed in Mean ± SD.

Results

Lipid Profile

Total cholesterol (TC), Triglyceride (TG) and High density-lipoprotein (HDL) decreased significantly before the administration of *Luffa aegyptiaca*on aqueous leaf extract on the diabetic rats (Table 1). The administration of the extracts at 100 mg, 200 mg and 300 mg/kg restored the alterations in lipid metabolism towards normalization. The extracts are effective as the normal drug metformin used in this research (Table 1).

Haematological indices

There was a noticeable decrease in PCV, Hb, and RBC levels of the positive control animals when related to the normal group (Table 2). Again, TWBC, MCH, MCHC and MCV increased significantly in the diabetic or positive control group. However, upon the extract administration the PCV, RBC and haemoglobin levels increased significantly across the treated animals when compared with the positive control (Table 2).

Liver Biomolecules

Serum total protein (STP), albumin (ALB) and amylase of the positive group reduced significantly when related with the normal control. The administration of the extract increased the levels of STP and ALB. Also the activity of serum amylase in the treated groups increased significantly when compared with the diabetic control group ($P<0.05$).

Table 1. Lipid profile, artherogenic and cardiovascular risk indices in diabetic rats treated with aqueous extract of *L. aegyptica.*

GROUP	TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	AI	CRI
NC	180.33 ± 2.89^{a}	238.67± 8.08^{a}	40.67 ± 16.2^{a}	91.93 ± 4.04^{a}	5.86±3.74^{a}	0.77 ± 0.22^{a}
DC	256.67 ± 32.1^{b}	195.67± 5.77^{b}	23.67 ± 1.53^{b}	193.87 ± 2.52^{b}	8.27 ± 0.71^{b}	0.92 ± 0.03^{b}
STD	186.67 ± 5.77^{c}	221.33 ±30.1^{c}	45.00 ± 5.00^{c}	97.67 ± 4.93^{c}	4.92 ± 1.23^{c}	0.69 ± 0.11^{c}
E1	205.00 ± 3.61^{d}	197.67± 2.52^{d}	34.33 ± 1.15^{d}	131.33 ± 3.06^{d}	5.76 ± 0.13^{c}	0.76 ± 0.01^{c}
E2	199.00 ± 1.73^{d}	205.00± 5.00^{d}	34.33 ± 1.53^{d}	123.67 ± 2.08^{e}	5.97 ± 0.21^{c}	0.78 ± 0.01^{c}
E3	182.67 ± 3.06^{c}	220.00 ±10.0^{d}	33.00 ± 2.00^{d}	112.33 ± 11.4^{f}	6.67 ± 0.38^{a}	0.82 ± 0.02^{a}

TC, Total cholesterol level; TG, Triglyceride level; HDL, High density-lipoprotein cholesterol; LDL, Low density-lipoprotein cholesterol; AI, Atherogenic index; CRI, Cardiovascular risk index. Values on the same column with the same letter superscript showed no major difference (P>0.05) while values on the same column with different letter superscripts indicated meaningful difference (P<0.05).

Table 2. Haematological indices of diabetic rats treated with aqueous extract of *L. aegyptica*.

GROUP	PCV (%)	Hb (g/l)	RBC ($\times10^{12}$/L)	TWBC ($\times10^{12}$/L)	MCH (pg/cell)	MCHC (%)	MCV (fL)
NC	38.33± 2.08^{a}	12.94± 0.42^{a}	4.60±0.10^{a}	8.27±0.85^{a}	28.14 ± 0.31^{a}	33.79± 0.80^{a}	83.40 ± 2.86^{a}
DC	27.33± 1.53^{b}	8.567± 0.67^{b}	2.63±0.15^{b}	11.50±1.00^{b}	34.44 ± 1.07^{b}	32.24± 1.11^{a}	103.51± 1.06^{b}
STD	30.00± 3.00^{c}	10.00± 1.00^{c}	4.93±0.51^{a}	8.23±0.45^{a}	21.67 ± 1.53^{c}	31.44± 1.17^{a}	64.00 ± 3.61^{c}
E1	38.00± 1.00^{a}	12.60± 0.67^{a}	3.40±0.10^{c}	11.37±0.86^{b}	32.53 ± 0.64^{d}	30.41± 0.78^{a}	103.19± 10.5^{b}
E2	36.00± 1.00a	12.33± 0.58^{a}	3.30±0.10^{c}	12.53±1.10^{b}	34.55 ± 0.97^{d}	29.83±0.52^{a}	102.83± 2.72^{d}
E3	27.00± 1.00^{b}	8.00 ± 1.00^{b}	4.40±0.10^{a}	7.33±0.21^{c}	20.33 ± 1.53^{a}	27.34± 0.89^{a}	67.67 ± 2.08^{c}

PCV, Packed cell volume; Hb, Haemoglobin concentration; RBC, Red blood cells; TWBC, Total white blood cells; MCH, mean value of corpuscular haemoglobin; MCHC, mean value of corpuscular haemoglobin concentration; MCV, mean value of corpuscular volume. Values on the same column with the same letter superscript showed no major difference (P>0.05) while values on the same column with different letter superscripts indicated meaningful difference (P<0.05).

Table 3.Total serum protein, albumin and serum amylase levels in diabetic rats treated with aqueous extract of *L. aegyptica.*

GROUP	TP (mg/dl)	ALB (mg/dl)	Serum amylase (U/L)
NC	6.27 ± 7.07^{a}	5.20 ± 0.85^{a}	5.03 ± 0.38^{a}
DC	4.10 ± 0.17^{b}	3.07 ± 0.06^{b}	3.13 ± 0.15^{b}
STD	7.83 ± 0.58^{b}	4.17 ± 0.06^{c}	4.90 ± 0.00^{c}
E1	10.10 ± 0.44^{c}	4.47 ± 0.21^{c}	7.87 ± 0.06^{d}
E2	11.20 ± 1.00^{c}	4.23 ± 0.21^{c}	6.30 ± 0.30^{c}
E3	15.20 ± 0.53^{d}	4.20 ± 0.20^{c}	3.17 ± 0.06^{b}

TP, Total protein level; ALB, Albumin level. Values on the same column with the same letter superscript showed no major difference (P>0.05) while values on the same column with different letter superscripts indicated meaningful difference (P<0.05).

Discussion

Ethnobotanical information shows that more than 800 plants are used as traditional remedies for diabetes treatment due to their efficacy, less side effects and low cost [10,11]. Plant extracts or individual phytochemical or group of phytochemicals has exhibited many mechanisms to reduce the diabetes status [10]. These extracts decreased or increased or stimulate the number of reactions involved in the reduction of the risks of the diabetes in animal experiments [10]. Established on these evidences, all the extracts of the plant described may have or no similar mechanisms of action in inhibiting diabetes in experimental animals. The plant extracts have evidence that they improved the various organs damaged due to diabetes. The extracts also have ability to change the structure and functions of affected parts [12]. A lot of publications have shown that some plant extracts led to body weight loss, possess anti-diabetic activity, reduction in serum cholesterol, serum triglyceride, total protein and blood urea and salvage in liver glycogen content. Insulin is secreted in pancreatic

β-cells in response to increase in postprandial blood glucose level (PBGL). Glucose is the nutrient accountable for insulin secretion and the process is called glucose stimulated insulin secretion. Disorder of glucose metabolism is a key factor in the pathogenesis of diabetes [9].

Lipid profile is another biochemical factor considered in the diagnosis of diabetes. The levels of total cholesterol, triglyceride, high density-lipoprotein, low density-lipoprotein, atherogenic and coronary risk index are pointer to the status of living organism [13]. High amount of lipids (Low density-lipoprotein) known as hyperlipidaemia is one of the indications of diabetes. Total cholesterol (TC), Triglyceride (TG) and High density-lipoprotein (HDL) decreases significantly before the injection of *Luffa aegyptiaca* aqueous leaf extract on the diabetic rats (Table 1). This arises from hypolipidaemia which is a likeness of high LDL, AI and CRI. This confirms diabetic conditions as shown in Table 1. On the administration of the extracts at 100 mg, 200 mg and 300 mg/kg the unit of alterations in lipid metabolism was restored towards normalization. The aqueous extracts when matched with the usual drug metformin used as a control in this research are in the same level of action towards stabilization of lipid metabolism in diabetic rats (Table 1). This report is in line with the work of Masudul *et al*. [9].

Diabetes reduces the immunomodulatory activity in diabetic conditions [14]. This resulted in low packed cell volume (PCV), red blood cell and haemoglobin. Therefore, low levels of the above mentioned indices are possible symptoms of diabetic disorder. There was a remarkable decrease in PCV, Hb, and RBC levels of the positive control animals when correlated with the normal group (Table 2,). Similarly, TWBC, MCH, MCHC and MCV increased significantly in the positive control group due to increased cell proliferation and metabolic stress in a diabetic animal. The observed increase probably indicates a shift in the mechanism of the defence of the cell which triggers increase in white blood cell and haemoglobin synthesis that results to anaemia and eventually a weakened defence system. This is the hallmark of diabetes. However, upon administration of the extracts, PCV, RBC and haemoglobin levels increased significantly across the treated animals when compared with the diabetic control [14]. Also the immunomodulatory activity of the diabetic rats was restored by a relative decrease in the levels of the proliferated TWBC, MCH, MCHC and MCV upon administration of the extracts [14], which is in agreement with this study.

The liver is a pivotal organ where constant oxidation and distribution of metabolite occurs. The liver also has regenerative ability (turnover rate) so that it can replace metabolites which are required by the cell at every given time. In diabetic conditions, the integrity of the liver is destroyed which hampers the effective performance of the hepatocytes. Serum total protein (STP), albumin and amylase of the positive group reduced significantly when compared with the normal control. This implies that the integrity of the liver has been compromised in alloxan treatment which eventually led to hypoamylasemia [15,16]. The treatment of the diabetic rats with the extract increased the levels of STP and ALB. Also, serum amylase activity of the treated groups increased significantly when compared with the diabetic control group ($P<0.05$). This shows that the liver integrity has been restored towards normalization which is consistent with the reports of Iweala *et al*. [8].

In conclusion, *Luffa aegyptiaca* aqueous leaf extract is shown to be effective as metformin in the treatment of diabetes evidenced by its restoration ability for deranged lipid metabolism as well as the normalization of the changes in the functions of liver and haematological indices. It therefore possesses protective effect on the liver and adipose tissue metabolism.

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