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Effect of Oral Administration of *Lasianthera africana* Leaf Extract on the Body Weight, Haematological Parameters and Lipid Profile in Alloxan-Induced Diabetic Rats

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KEYWORDS

Lasianthera africana, Diabetic rats, Lipid levels, Haematological parameters

A B S T R A C T

Aqueous extract of *Lasianthera africana* leaf was orally administered daily to alloxan (150mg/kg; ip)-induced diabetic rat for 28 days at doses of 50mg/kg, 100mg/kg and 200mg/kg and the effects on the body and organs weight, haematological parameters, blood glucose and serum lipid levels were investigated. The treatment's effects were compared with three controls (normal, diabetic and diabetic treated daily with a standard anti-diabetic drug (metformin at 100mg/kg)). The diabetic control rats showed significant ($p<.0.5$) body weight reduction, varying haematological abnormalities, significantly ($p<0.05$) higher fasting blood glucose, total cholesterol, triglycerides, LDL-cholesterol and significantly ($p<0.05$) lower HDL-cholesterol when compared with normal control rats. Treatment of diabetic rats with doses of the leaf extract resulted in loss weight recovery, varying amelioration of haematological abnormalities, significantly ($p<0.05$) lower levels of fasting blood glucose, total cholesterol, triglycerides, LDL-cholesterol and significantly ($p<0.05$) higher HDL-cholesterol when compared with the diabetic control rats. The effects of the extract were comparable with metformin. The results suggest that L. africana leaf extract is a potent hypoglycaemic and hypolipidaemic agent and is capable of normalizing haematological abnormalities associated with diabetes mellitus.

Introduction

Diabetes mellitus (DM) is a group of metabolic diseases characterized by chronic hyperglycaemia resulting from defects in insulin metabolism and impaired function in carbohydrate, lipid and protein metabolism that leads to long-term complications (Sepici-Dincel et al., 2007). Among the complications associated with diabetes mellitus include hyperlipidaemia (Tang et al., 2006; Akah et al., 2009) and haematological abnormalities (Saba et al., 2010; Azeez at al., 2010).

The WHO (2004) report estimated that 1.7 million people in Nigeria had diabetes with the projection that the number will triple by 2030. Pharmacological treatment of diabetes mellitus is based on oral hypoglycaemic agents and insulin injection which have so many side effects (Zhang et al., 2005), coupled with its high cost which are not affordable in poor economic communities. Consequently, in rural parts of worldwide societies, traditional remedies from plant sources with minimal side effect are frequently employed to manage the disorder (Sepici-Dincel et al., 2007).

Lasianthera africana is a perennial, glabrous, shrub that belongs to the family lcacinaceae. It is called "editan" in Efik and Ibibio local dialects of Nigeria. It grows up to a height of 61 – 136cm (Hutchinson and Dalziel, 1973). Among the ibibios, four local varieties ("afia", "obubit", "akai" and "idim") distinguished by their taste, leaf colour and ecological distribution are known (Bassey et al., 2006). Traditionally, the leaves of all ethno-varieties are utilized for both food and therapeutic purposes especially in rural communities where they are mostly found. According to Andy et al. (2008), the plant has been exploited since pre-historic time by traditional herbalists for the treatment of various ailments including typhoid fever, diarrhoea and candidiasis. Bassey et al. (2006), reported the present of substantial levels of alkaloids, flavonoids, terpenes, saponins, anthraquiniones, phlobatannins, cardiac glycosides and tannins in the leaf.

One unique characteristic of the leaf is that it has bitter taste. Some leafy vegetables with bitter taste have been implicated in enhancing insulin production in experimental diabetic rats and have potentials for diabetic control and management (Akah et al., 2002). The present study was aimed at determining the effect of administering *Lasianthera africana* leaf extract on the body weight, haematological indices and lipid profile in alloxan-induced diabetic rats.

Materials and Methods

Twigs of *Lasianthera africana* ("afia" variety) were harvested from a garden at Aka Offot in Uyo Local Government Area of Akwa Ibom State, Nigeria and authenticated at the Taxanomy Unit of the Department of Botany and Ecological Science, University of Uyo. The leaves were destalked, washed in potable water, spread under shade to air dry, cut (2mm width) and blended with water (1:3 w/v) using kenwood blender (Kenwood Ltd., Havant, UK). The blend was left for 2h at room temperature (26±2oC) before filtering through 425 micrometer pore size sieve. The filtrate was stored at 4oC for subsequent use. A known volume of the filtrate was evaporated to dryness in a conventional oven (model P.P. 22 US, Genlab, England) and the weight of the residue was used to determine the concentration of the filtrate which was in turn used to determine the dose of administration of the extract (Ikewuchi and Ikewuchi, 2012).

Animal procurement and care

Three months old male albino rats weighing between 138 and 179g obtained from the Animal Breeding Unit, Faculty of Basic Medical Sciences, University of Uyo, Akwa Ibom State, Nigeria were used for the experiment. Animals were housed in well ventilated stainless steel cages containing wood shavings for bedding. The animals were allowed to acclimatize for 7 days and maintained with standard grower's mash (UAC Vital Feed produced by Grand Cereals, Jos, Nigeria) and tap water ad libitum prior to experimentation. Animals were maintained under normal environmental temperature (26±2oC) with normal 12:12 hour dark/light cycle. The experiment was conducted in accordance with the internationally accepted principles for Laboratory Animal use and care as found in the US guidelines (NIH Publication No. 85-23, revised in 1985).

Inducement of diabetes

Animals were allowed to fast for 16h and diabetes mellitus was induced by intra-peritoneal injection with freshly prepared alloxan monohydrate (Sigma Aldrich Co., USA) in distilled water in a dose of 150mg/kg body weight (Antai et al., 2010). Initial blood glucose was determined prior to inducement with alloxan and after 7 days of inducement to confirm diabetic state of the rats. Blood was collected from the tip of tail vein for glucose determination. Rats with fasting blood glucose levels above 230mg/dl were selected for the study.

Experimental protocol

Twenty-five (25) alloxan-induced diabetic male albino rats were divided into five groups (groups 2 – 6) of five rats per group. Three groups served as control (group 1 (normal control), group 2 (diabetic control) and group 3 (diabetic treated with a standard anti-diabetic drug, metformin at a dose level of 100mg/kg daily for 28 days (Tang et al., 2006)). Rats in groups 4, 5 and 6 were orally given aqueous extract of *L. africana* (using canula) at doses of 50, 100 and 200mg/kg body weight respectively, per day for 28 successive days. Rats in all the groups had access to feed and drinking water ad libitum for the 28 days. At the end of the treatment, animals were fasted overnight, but allowed access to water ad libitum. The rats were euthanized and ex-sanguinated under chloroform anaesthesia and their blood collected by jugular vein puncture (Wilson et al., 2001) and fasting blood glucose measured. Part of the blood samples were dispensed into ethylene-diamine-tetra-acetic acid (EDTA) coagulant bottles for the haematological analysis. The remaining portions were dispensed into sterile plain bottles, allowed to stand for 3 hours at room temperature (26oC) to ensure complete clotting and centrifuged at 3500 rpm for 10 minutes. The clear sera were aspirated off and stored at -20oC for lipid profile determination.

Methods of analysis

Measurement of weight of rats: The weight of each rat, liver and kidneys were measured using Ohaus Electronic weighing balance (Model CS 2000, USA).

Determination of haematological indices

The red blood cell (RBC) count, white blood cell (WBC) count, haemoglobin (HGB), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobiin concentration (MCHC) were determined using automated haematology analyzer (SYSTEM KX-21N^{TM}, Japan).

Assay of biochemial indices

Blood glucose levels were measured using One Touch Ultra 2 blood glucose meter (Lifescan Inc., Milpitas, USA). Serum total cholesterol (TC), triglyceride (TG) and high density lipoprotein cholesterol (HDL-c) were determined using standard ready-to-use reagent kits (Randox Ltd., UK) following the manufacturer's instructions. Low density lipoprotein cholesterol (LDL-c) was calculated using Freidewalds formula as reported by Sood (2006).

Statistical analysis

The results of the studies were expressed as mean ±SD (standard deviation) of triplicate determinations. The data obtained were subjected to one-way Analysis of Variance (ANOVA) using SPSS version 18 statistical software package (SPSS, Inc., USA) to determine variation between means. Tukey's test was used for multiple comparisons. Significant variation was accepted at $p<0.05$.

Results and Discussion

Effect on body and organ weights

Table 1 shows the body weight of normal control, diabetic control and treated diabetic rats at the commencement of the study (day 0) and termination of the study (day 28). The result showed that normal control rats had significant ($p<0.05$) weight gain (15% weight gain) on day 28. Diabetic control rats however exhibited significant ($p<0.05$) weight reduction (15.30% weight loss) on day 28 when compared with day 0. Diabetic rats treated with standard antidiabetic drug (metformin) and doses of *Lasianthera africana* leaf extract significantly ($p<0.05$) regained their weight on day 28 when compared with day 0. The untreated diabetic rats had the highest relative liver and kidney weights. However, the values were not significantly ($p>0.05$) different from the normal control and treated diabetic groups (Table 2).

Weight loss as observed in diabetic control rats (Table 1) is a prominent symptom of diabetes (Nair *et al., 2006;* Antai *et al.,* 2010). The liver, during impaired glucose utilization breaks down muscle glycogen, protein and fat stores of the body for the generation of glucose to meet body's need. A further combination of polyuria, retarded growth and repair process leads to emaciation and weight loss (Guyton and Hall, 2004). The general regain in body weight of the diabetic rats treated with doses of *L. africana* leaf extract (Table 1) suggest that the extract did not induce anorexia, an effect that could have resulted to loss of weight. The lower values of the relative kidney and liver weights of the treated diabetic rats relative to the diabetic control rats (Table 2) may be due to the ability of the heterogeneous phytoconstituents in the crude extract to produce synergistic effect on the alloxan mediated free radical activities (Mazunder *et al.,* 2005). Bassey *et al.* (2006) identified flavonoid as one of the bioactive compounds present in *L. africana* leaf and flavonoid has been associated with antioxidant and free radical scavenging activities (Hillwell, 1994).

Effect on Haematological Indices

The effect of oral administration of doses of *Lasianthera africana* leaf extract on haematological parameters in alloxan-diabetic rats is shown in Table 3. Alloxan-induced diabetes caused significant ($p<0.05$) reductions in haemoglobin concentration and mean corpuscular haemoglobin concentration and non significance ($p>0.05$) reductions in red blood cell count, haematocrit, mean corpuscular volume and mean corpuscular hemoglobin in untreated

diabetic rats when compared with the normal control group. The white blood cell count was however non significantly ($p>0.05$) higher in untreated diabetic rats than in the normal control rats. Treatment of diabetic rats with either metformin (a standard antidiabetic drug) or doses of *Lasianthera africana* leaf extract led to significant ($p<0.05$) increases in haemoglobin concentration, haematocrit and mean corpuscular haemoglobin concentration and non significance ($p>0.05$) increases in red blood cell count, mean corpuscular volume and mean corpuscular haemoglobin in the treated diabetic rats when compared with the diabetic control rats. The treatments also led to non significance ($p>0.05$) reduction in white blood cell count in the treated diabetic groups when compared with untreated diabetic rats.

The haematological abnormalities observed in diabetic control rats relative to the normal control rats (Table 3) could be due to the effects of alloxan on rapidly dividing haemopoietic cells and suppression of haemopoiesis as a result of insulin deficiency occasioned by the selective destruction of the β cells in the Islets of Langerhans of the pancreas by alloxan (Ruxue *et al.*, 2004; Philips *et al.*, 2004). The result obtained in this study is in agreement with the reports by Azeez *et al.* (2010) and Ikewuchi and Ikewuchi (2012). Shevchenko and Elfimov (1995) had earlier attributed decreases in HGB, HCT, MCH and MCHC in glomectomized diabetic rats to the suppression of haemopoiesis in diabetes. Following treatment of the diabetic rats with doses of *L. africana* leaf extract, the normocytic hypochronic anaemia observed in the diabetic control rats as a result of reduction of HGB, HCT, MCH and MCHC was corrected by the extract. Similar results were reported by Akah *et al.* (2009) and Ikewuchi and Ikewuchi (2012) for *Vernonia amygdalina* leaf extract and extract of rhizomes of *Sansevieria senegambica*, respectively. An elevated white blood cell count in peripheral blood is a known risk factor of coronary artery disease (Takeda *et al.*, 2003). The reduced white blood cell count found in the treated diabetic rats is an indication of the ability of the leaf extract to protect against diabetic-induced increases in total white blood cell counts and reduction of the risk of coronary artery disease.

Effect on fasting blood glucose and lipid profile

The fasting blood glucose, total cholesterol, triglycerides, LDL- cholesterol and LDL/HDL-cholesterol ratio of the diabetic control rats were significantly ($p<0.05$) higher than the values found in the normal control and treated diabetic groups (Table 4). On the other hand, the serum HDL-cholesterol and HDL/TC ratio of the diabetic control rats were significantly ($p<0.05$) lower than the values found in the normal control and treated diabetic groups. Treatment of diabetic rats with *L. africana* leaf extract at doses of 50, 100 and 200mg/kg body weight for 28 days resulted in significant ($p<0.05$) decrease in blood glucose by 71.36, 76.87 and 70.57%; in total cholesterol by 39.42, 43.11 and 44.38%; in triglycerides by 33.75, 36.06 and 35.41% and in LDL-cholesterol by 58.54, 60.93 and 62.92%, respectively, compared to untreated diabetic group. The LDL/HDL ratios of the treated diabetic groups were significantly ($p<0.05$) lower than that of the untreated diabetic group. Diabetic rats treated with doses of the leaf extract had significantly ($p<0.05$) higher levels of HDL-cholesterol and HDL/TC ratios relative to diabetic control group (Table 4). The observed

ameliorative effects of the leaf extract were significantly better than those of metformin.

The results demonstrated that *L. africana* leaf extract exhibited anti-diabetic properties by significantly ($p<0.05$) lowering blood glucose in the treated diabetic rats when compared with diabetic control group (Table 4). The blood glucose lowering effect was more pronounced in the group that received 100mg/kg body weight of the extract than the effects of 200mg/kg body weight of the extract or metformin. Similar low hypoglycaemic response at higher dose administration of some plant products have been reported by Kesari *et al.* (2005) and Mowla *et al.* (2009) for *Murraya koenigii* and *Trigonella foenum-graecum* seed extract, respectively.

The observed abnormalities in the serum lipid profile of diabetic control rats could be attributed to uninhibited action of lipolytic hormones on the peripheral fat depots (Pari and Latha, 2002, Rajagopal and Sasikala, 2008). These results are in accordance with the findings of Tang *et al.* (2006) and Ikewuchi and Ikewuchi (2012) who reported marked increases in serum cholesterol, triglycerides and LDL-cholesterol levels and decreased HDL-cholesterol in alloxan and streptozotocin-induced diabetic rats. Significant ($p<0.05$) reduction in the serum total cholesterol, triglycerides and LDL-cholesterol and significant ($p<0.05$) increased in the HDL-cholesterol in the treated diabetic rats when compared with the diabetic control rats are important findings in this study since decreased levels of cholesterol and total lipids minimize the incidence of many cardiovascular complications in diabetes (Bersot *et al.*, 2003; Shen, 2007). The ameliorative effect of the extract could be attributed to the heterogeneous phytoconstituents in the crude extract.

Table.1 Effect of doses of *Lasianthera africana* leaf extract on body weight of diabetic rats treated for 28 days (g)

Treatment Groups	Dose (mg/kg)	Weight before Inducement	7 Days after Inducement (Day 0)	Final Weight (Day 28)
Normal control	-	138.00±1.58	140.00±1.58	167.00[a]±2.55
Diabetic control	-	175.20±2.58	160.80±2.68	136.20±2.39
Diabetic + Metformin	100	179.00±3.39	157.80±2.17	159.00±2.24
Diabetic + Extract	50	175.00±2.92	162.80±2.72	168.00±3.56
Diabetic + Extract	100	173.00±3.12	160.00±2.55	167.00±3.81
Diabetic + Extract	200	162.80±3.53	147.20±3.19	151.00±2.33

Values are Means ± SD, n = 5. Means on the same row with different superscripts are significantly different at $p<0.05$

a = $p<0.05$ (compare with Day 0).

Table.2 Effect of doses of *Lasianthera africana* leaf extract on absolute and relative organ weight of diabetic rats after 28 days of treatment

Treatment Groups	Dose (mg/kg)	Absolute Liver Weight (g)	Relative Liver Weight (g/100g)	Absolute Kidney Weight (g)	Relative Kidney Weight (g/100g)
Normal control	-	4.86±1.09	3.01	0.94±0.03	0.58
Diabetic control	-	5.66±0.05	4.16	1.12±0.04	0.82
Diabetic + Metformin	100	6.45±0.07	4.06	1.18±0.04	0.74
Diabetic + Extract	50	6.87±0.05	4.09	1.28±0.04	0.76
Diabetic + Extract	100	6.79±0.06	4.07	1.31±0.04	0.78
Diabetic + Extract	200	5.79±0.02	3.97	1.09±0.04	0.75

Values are Means ± SD, n = 5.

Table.3 Effect of doses of *Lasianthera africana* leaf extract on haematological indices of diabetic rats after 28 days treatment

Parameters	Normal Control	Diabetic Control	Diabetic + Metformin (100mg/kg)	Diabetic + Extract (50mg/kg)	Diatetic + Extract (100mg/kg)	Diabetic + Extract (200mg/kg)
WBC ($X10^3$/µl)	15.23±2.15	16.40±3.94	13.86±1.71	14.83±1.86	15.71±1.95	13.98±0.97
RBC ($X10^b$/µl)	7.25±0.43	6.52±0.56	7.78±0.19	7.82±0.40	7.79±0.68	7.97^b±0.28
HGB (g/dl)	13.90±0.93	10.14^a±3.69	14.37^b±0.39	14.33^b±0.43	14.71^b±0.34	14.53^b±0.59
HCT (%)	40.89±1.79	31.44±3.69	46.10^b±2.95	45.19^b±3.18	45.71^b±0.91	45.24^b±3.02
MCV (fl)	55.90±0.29	54.11±1.22	56.83±1.81	57.60±0.83	55.85±1.11	56.71±1.53
MCH (pg)	18.70±0.21	17.80±0.45	18.40±0.43	18.30±0.33	18.23±0.32	18.25±0.36
MCHC (g/dl)	33.40±0.90	29.90^a±0.53	32.27^{ab}±0.59	32.70^b±0.22	32.73^b±0.12	32.65^b±0.19

Values are Means ± SD, n = 5. Means on the same row with different superscripts are significantly different at $p<0.05$.

a = $p<0.05$ (Test groups compared with normal group); b = $p<0.05$ (Compared with diabetic control)

c = $p<0.05$ (Compared with diabetic + metformin)

Table.4 Effect of doses of *Lasianthera africana* leaf extract on blood glucose and lipid profile of diabetic rats after 28 days treatment

Parameters	Normal Control	Diabetic Control	Diabetic + Metformin (100mg/kg)	Diabetic + Extract (50mg/kg)	Diatetic + Extract (100mg/kg)	Diabetic + Extract (200mg/kg)
Blood glucose (mg/dl)	67.00±2.12	377.80^{a}±6.14	101.60^{ab}±3.97	108.20^{ab}±5.26	87.40^{abc}±4.72	111.20^{ab}±3.89
Total cholesterol (mg/dl)	99.21±0.92	194.11^{a}±0.96	139.80^{ab}±1.77	117.59^{abc}±3.34	110.43^{abc}±1.79	107.97^{abc}±1.95
Triglyceride (mg/dl)	88.93±2.09	182.56^{a}±2.47	131.19^{ab}±1.77	120.00^{abc}±1.92	116.73^{abc}±2.53	117.91^{abc}±2.38
HDL – chol (mg/dl)	29.05±1.23	19.41^{a}±0.85	23.01^{a}±1.02	36.11^{abc}±1.16	33.09^{bc}±2.19	32.47^{bc}±2.17
LDL – chol (mg/dl)	52.37±1.81	138.19^{a}±2.67	90.55^{ab}±3.39	57.29^{bc}±1.86	53.99^{bc}±3.15	51.92^{bc}±2.91
HDL/TC	0.29±0.01	0.10^{a}±0.00	0.17^{ab}±0.01	0.31^{bc}±0.01	0.30^{bc}±0.00	0.30^{bc}±0.02
LDL/HDL	1.80±0.03	7.1^{a}±0.44	3.94^{ab}±0.31	1.59^{bc}±0.09	1.64^{bc}±0.19	1.61^{bc}±0.13

Values are Means ± SD, n = 5. Means on the same row with different superscripts are significantly different at $p<0.05$.
a = $p<0.05$ (Test groups compared with normal group); b = $p<0.05$ (Compared with diabetic control)
c = $p<0.05$ (Compared with diabetic + metformin)

Conclusion

The study shows that *Lasianthera africana* leaf extract has potential hypoglycaemic action and is capable of ameliorating the effects of alloxan-indued diabetes on haematological parameters and lipid levels in rats. Further biochemical investigations are needed to elucidate the mechanism of action of this extract.

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