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Original Research Article

Ameliorative Effects of *Lasianthera africana* Leaf Powder on Haematological and Biochemical Parameters in Alloxan–Induced Diabetic Rats

U.E. Inyang[1]* and J.C. Ani[2]

[1]Department of Food Science and Technology, University of Uyo, Uyo, Akwa Ibom State, Nigeria
[2]Department of Food Science and Technology, University of Nigeria, Nsukka, Enugu State, Nigeria

**Corresponding author.*

Abstract

The ameliorative effective of doses of *Lasianthera africana* leaf powder on haematological and biochemical parameters in alloxan-induced diabetic rats was investigated. The experiment included one normal control group, one diabetic control group, one diabetic group treated with a standard antidiabetic drug (metformin at 100mg/kg) and three diabetic groups treated with doses (0.25, 0.50 and 1.00g/kg) of *L. africana* leaf powder for 28 days. Each group consisted of 5 rats. Rats in diabetic control group exhibited abnormal haematological parameters; significant ($p<0.05$) increases in AST, ALT, ALP, and creatinine; marginal increase in uric acid; significant ($p<0.05$) decreases in total proteins and albumin and marginal ($p>0.05$) decrease in globulin when compared with normal control group. Chronic oral administration of doses of *L. africana* leaf powder to alloxan diabetic rats led to the restoration of the haematological parameters to the values obtained in normal control rats. The treatment also led to significant ($p<0.05$) decreases in AST, ALT, ALP and creatinine; marginal decrease in uric acid and significant ($p<0.05$) increases in total protein and albumin when compared with the diabetic control rats. The ameliorative effect of the leaf powder on the haematological and biochemical parameters was comparable to that of metformin (a standard anti-diabetic drug). The study has clearly demonstrated that *L. africana* leaf powder is capable of normalizing haematological and biochemical abnormalities associated with the diabetes mellitus.

Keywords

Alloxan diabetic rats
Haematological parameters
Hepatoprotective effect
Lasianthera africana
Leaf powder
Renal function indices

Introduction

Diabetes mellitus (DM) is a chronic disorder of carbohydrate, fat and protein metabolism characterized by elevation of both fasting and post-paradinal blood sugar levels (Kesari et al., 2005). Several studies on enzymes involved in hepatic glucose metabolism in rats with alloxan and streptozotocin diabetes have

shown an increased in the activity of gluconeogenic enzymes including aminotransferases (Rawi et al., 1998). Development of normocytic hypochronic anaemia as a result of significant reduction in haemoglobin (Hb) concentration, packed cell volume (PCV) and red blood cell (RBC) counts has been reported (Azeez et al., 2010; Saba et al., 2010).

Food has traditionally been viewed as a means of providing nutrients for normal growth and development. Today, science has however clearly demonstrated additional role of some foods in reducing disease risk, and consumers have learned that food has a greater impact on health than previously known. A new self-care paradigm recognizes that food can provide health benefits that can co-exist with traditional medical approaches to disease treatment (Mishra and Tumnay, 2011). Green leafy vegetables are among the foods that play this dual role of providing nutrients and health benefits. According to Odukoya et al. (2007), high consumption of leafy vegetables has been associated with a lowered incidence of degenerative diseases. These protective effects are considered to be related to various anti-oxidants contained in the vegetables. Accessibility, affordability and with fewer side effects compared with synthetic drugs are some of the factors contributing to increased use of plant products in developing countries in the prevention or management of various metabolic disorders (Mowla et al., 2009).

Lasianthera africana is one of the green leafy vegetables in Akwa Ibom State, Nigeria that has been exploited by traditional herbalists for the treatment of various ailments including typhoid fever, diarrhoea, candidiasis, constipation and general stomach ache (Sofowora, 1989; Andy et al., 2008). It belongs to the family Icacinaceae. It is called "editan" by the Efik and Ibibio ethnic groups in Nigeria. It is a perennial glabrous shrub that reaches a height of 61-136cm (Hutchison and Dalziel, 1973). Among the Ibibios, four local varieties ("afia" (white), "obubit" (black), "idim" (riverine) and "akai" (forest)) distinguished by their taste, leaf colour and ecological distribution are known (Bassey et al., 2006).

The leaves of all ethno-varieties are eaten by the Ibibios. Ebana et al. (1996) reported that the leaves of *L. africana* are rich in chemical compounds of nutritional and medicinal importance. Bassey et al. (2006) reported that substantial levels of alkaloids, flavonoids, saponins, anthraquinones, tannins and glycosides are present in all the four ethno-varieties. The presence of some of these bioactive compounds in addition to the other basic nutrients in the leaves confirms that they possess medicinal properties and could be useful in the management of some diet related chronic diseases. This present study was aimed at assessing the effect of the leaf powder administration on the haematological and biochemical parameters in alloxan induced diabetic rats.

Materials and methods

Twigs of *L. africana* (white 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, Nigeria. The leaves were destalked, washed in potable water, spread under shade to air dry, cut (2mm width), oven dried (50°C) in a conventional air oven (Model P. P. 22 US, Genlab, England) for 36 h, milled and sieved to pass through 425 micrometer pore size sieve to obtain the leaf powder. The powder was stored at 4°C for subsequent use.

Animal procurement and care

Three months old male albino rats weighting between 138 and 180g obtained from the Animal Breeding Unit, 'Faculty of Basic Medical Sciences, University of Uyo, Akwa Ibom State, Nigeria were used for the study. Animals were housed in well ventilated stainless steel cages containing wood shavings for bedding. The rats were allowed to acclimatize for 7 days and maintained with standard growers mash (UAC vital feed produced by Grand cereals, Jos) and tap water *ad libitum* prior to experimentation.

Animals were maintained under normal environmental temperature (26±2°C) with normal 12:12 h dark/light cycle. The room was cleaned and disinfected regularly. Soiled wood shavings were replaced weekly. The feed and water containers were also washed regularly. Each rat was marked for identification. 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

Alloxan monohydrate (Sigma-Aldrich Co., USA) was used to induce diabetes mellitus in normoglycaemic rats. Animals were allowed to fast for 16 h and were injected intraperitonially (i.p.) with freshly prepared alloxan monohydrate in distilled water in a dose of 150mg/kg body weight (Antai et al., 2010). Initial blood glucose was determined before the inducement with alloxan. Fasting blood glucose (FBG) was determined after 7 days of inducement to confirm diabetic state of the rats. Rats showing fasting blood glucose levels above 230mg/dl were selected for the study.

Experimental protocol

Twenty-five (25) alloxan-induced diabetic male rats were divided into five groups (groups 2 – 6) of five rats per group. Rats in group one (normal control) were given feed and drinking water only for 28 days. Rats in group 2 (diabetic control) were given feed and drinking water only for 28 days. Rats in group 3 were given feed, drinking water and treated with metformin (a standard anti-diabetic drug) at a dose level of 100mg/kg body weight daily (Tang et al., 2006) for 28 days.

Rats in groups 4, 5 and 6 were treated daily with doses (0.25, 0.50 and 1.00g/kg body weight) of the leaf powder, respectively and were also given feed and drinking water for 28 days. The leaf powder dispersed in 10ml of water was given to the rats by oral administration using canula. The first day of administration was taken as day zero while the sacrifice day was on day 28. 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 (FBG) was 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 (26^oC) to ensure complete clotting and centrifuged at 3500 rpm for 10 min. The clear sera were aspirated off and stored at -20^oC for biochemical evaluation.

Methods of analysis

Haematological indices

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

Assay of biochemical indices

Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), creatinine, uric acid, total proteins, and albumin were determined using standard ready-to-use reagent kits (Randox Ltd., UK) following the manufacturer's instructions. Globulin value was obtained by subtracting albumin from total protein (Sood, 2006).

Statistical analysis

The results of the studies were expressed as means ± 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 treatment means. Tukey's test was used for multiple comparisons. Significant variation was accepted at $p<0.05$.

Results

Effect of administration of *L. africana* leaf powder on haematological parameters of alloxan-induced diabetic rats

Table 1 shows the haematological parameters of alloxan-induced diabetic rats following treatment with doses of *L. africana* leaf powder and metformin (a standard anti-diabetic drug). The haemoglobin (HGB), haematocrit (HCT) and mean corpuscular haemoglobin concentration (MCHC) were significantly ($p<0.05$) lower while the red blood cell (RBC) count, mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) were marginally ($p>0.05$) lower in diabetic control rats than in the normal control rats. On the contrary, the white blood cell (WBC) count

was marginally (p>0.05) higher in diabetic control rats than in the normal control rats. Treatment of diabetic rats with either metformin or doses of *L. africana* leaf powder led to varying levels of restoration of these parameters towards the values obtained in normal control rats.

Table 1. Haematological parameters of alloxan-induced diabetic rats after 28 days treatment with *L. africana* leaf powder and metformin.

Parameters	Normal control	Diabetic control	Diabetic + metformin (100mg/kg)	Diabetic + leaf powder (0.25g/kg)	Diabetic + leaf powder (0.50g/kg)	Diabetic + leaf powder (1.00g/kg)
RBC ($\times10^6$.µl)	7.25±0.01	6.52±0.56	7.78±0.19	7.32±0.33	7.44±0.61	7.69±0.55
WBC ($\times10^3$.µl)	15.23±2.04	16.40±3.94	13.86±1.71	14.13±1.81	14.85±0.59	14.04±1.11
HGB (g/dl)	13.90±0.03	10.14^a±0.31	14.37^b±0.39	13.65^b ±0.67	13.66^b ±0.38	14.38^b±0.49
HCT (%)	40.89±1.01	31.44^a±3.69	46.10^b±2.95	40.30^b ±3.43	42.40^b ±2.95	41.67^b±0.71
MCV (fl)	55.90±0.28	54.11±1.22	56.83±1.83	56.10±1.64	55.00±1.11	56.70±1.53
MCH (pg)	18.70±0.14	17.80±0.45	18.41±0.43	18.40±0.27	18.65±0.47	18.63±0.19
MCHC (g/dl)	33.40±0.42	29.90^a±0.53	31.27^{ab}±0.59	31.95±0.19	32.70±0.34	32.10±0.22

Values are Means ± SD (standard deviation) of triplicate determinations. Means on the same row with different superscripts are significantly different at p<0.05.
a = p<0.05 (Test groups compared with Normal control group)
b = p<0.05 (Compared with diabetic control)
c. = p<0.05 (Compared with diabetic + metformin)

Effect of administration of *L. africana* leaf powder on the biochemical parameters in alloxan-induced diabetic rats

Table 2 shows the biochemical parameters of alloxan-induced diabetic rats following treatment with metformin (a standard anti-diabetic drug) and doses of *L. africana* leaf powder. The result shows that the activities of AST, ALT, ALP and creatinine were significantly (p<0.05) elevated by 67.82, 94.87, 107.70 and 33.3%, respectively while uric acid was marginally (p>0.05) elevated by9.97% in diabetic control rats when compared with the normal control rats. On the contrary, the total proteins and albumin were significantly (p<0.05) lower by 24.14 and 43.73%, respectively while globulin was marginally (p>0.05) lower by 1.54% in the diabetic control rats than in the normal control rats. Oral administration of *L. africana* leaf powder to alloxan-induced diabetic rats at doses of 0.25, 0.50 and 1.00g/kg body weight caused significant (p<0.05) reduction in the activities of AST by 19.79, 32.49 and 32.36%, ALT by 31.58, 40.01 and 43 26%, ALP by 31.16, 40.33 and 49.75% and creatinine by 16.18, 20.59 and 17.65% and marginal (p>0.05) reduction in the activities of uric acid by 8.35, 10.26 and 9.55%, respectively when compared with diabetic control rats. On the contrary, the treatment led to significant (p<0.05) increased in total proteins by 20.15, 36.72 and 30.89% and albumin by 63.03, 78.67 and 76.30% for diabetic rats that received 0.25, 0.50 and 1.00 g/kg body weight doses of the leaf powder, respectively. The ratios of albumin to globulin were higher in the treated diabetic rats and normal control rats than in the diabetic control rats. The effect of *L. africana* leaf powder on the biochemical parameters in alloxan-induced diabetic rats compared favourably with that of the metformin (a standard anti-diabetic drug).

Discussion

Literature has shown that ingestion of medicinal compounds or drugs can alter the normal range of haematological parameters (Ajagbonna et al., 1999). In this study, diabetes induced by alloxan was associated with normocytic hypochromic anaemia as a result of reduction in haemoglobin, haematocrit, red blood cell count, mean corpuscular volume, mean corpuscular

haemoglobin and mean corpuscular haemoglobin concentration (Table 1). This might be due to the effect of alloxan on rapidly dividing haemopoietic cells and suppression of haemopoiesis as a result of deficiency occasioned by the selective destruction of the β-cells in the Islets of Langerhans of the pancreas by alloxan (Ruxue et al., 2004). Some investigators have similarly reported decreases in red blood cell count, haemoglobin, haematocrit, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration in untreated diabetic rats when compared with normal control rats (Shevchenko and Elfimov, 2000; Azeez et al., 2010; Saba et al., 2010; Ikewuchi and Ikewuchi, 2012). Treatment of diabetic rats with doses of *L. africana* leaf powder resulted in significant (p<0.05) ameliorative effect on the aforementioned haematological parameters towards the values obtained in normal control rats. The effect of the leaf powder compared favourably with metformin (a standard anti-diabetic drug).

Table 2. Biochemical parameters of alloxan-induced diabetic rats after 28 days treatment with *L.africana* leaf powder and metformin.

Parameters	Normal Control	Diabetic Control	Diabetic + Metformin (100mg/kg)	Diabetic + Leaf Powder (0.25g/kg)	Diabetic + Leaf Powder (0.50g/kg)	Diabetic + Leaf Powder (1.00g/kg)
AST (IU/L)	39.09±0.38	65.60^{a}±0.56	49.51^{ab}±0.41	52.62^{abc}±0.33	44.29^{abc}±0.29	46.37^{abc}±0.23
ALT (IU/L)	26.50±0.47	51.64^{a}±3.94	38.14^{ab}±0.61	35.33±0.41	30.98^{abc}±0.60	29.30^{abc}±0.26
AIP (IU/L)	147.80±2.18	306.98^{a}±0.31	217.98^{ab}±3.35	211.33^{abc}±2.84	183.17^{abc}±3.61	154.25^{abc}±1.47
Creatinine (mg/dl)	0.51±0.02	0.68^{a}±3.69	0.53^{b}±0.04	0.57^{b}±0.01	0.54^{b} ±0.05	0.56^{b}±0.04
Uric acid (mg/dl)	3.81±0.17	4.19±0.53	3.76±0.29	3.84±0.32	3.76±0.31	3.79±0.25
Total protein (g/dl)	7.00±0.06	5.31^{a}±0.31	6.69^{b}±1.16	6.38^{b} ±0.29	7.26^{b} ±0.51	6.95^{b}±0.19
Albumin (g/dl)	3.75±0.17	2.11^{a}±3.69	3.83^{b}±0.33	3.44^{b} ±0.38	3.77^{b} ±0.36	3.72^{b}±0.11
Globulin (g/dl)	3.25±0.23	3.20±1.22	2.86±0.48	2.94±0.62	3.49±0.12	3.23±0.00
Albumin/ Globulin ratio	1.15	0.66	1.34	1.17	1.08	1.15

Values are Means ± SD (standard deviation) of triplicate determinations. Means on the same row with different superscripts are significantly different at p<0.05.

a = p<0.05 (Test groups compared with Normal control group)

b = p<0.05 (Compared with diabetic control)

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

Similar higher white blood cell count in untreated diabetic rats relative to normal control rats as obtained in this study had been reported by Akah et al. (2009). According to some experimental and pathological studies, white blood cell plays important roles in destabilizing coronary artery plaques at the onset of acute coronary syndrome (Moreno et al., 1994; Libby, 2001). However, an elevated white blood cell count in peripheral blood is a known risk factor of coronary artery disease (Takeda et al., 2003). Therefore, the lower white blood cell count obtained in the diabetic treated rats implied the ability of the leaf powder to protect against diabetic-induced increases in total white blood cell count and reduction of the risk of coronary artery disease. The heterogeneous phytochemicals in the leaf powder may have contributed to the observed effect on haematological parameters. However, further work may be required to elucidate the exact mechanism of action and the specific constituents responsible for the observed effect.

The present study demonstrated that alloxan-induced diabetic rats exhibited significant (p<0.05) elevated levels of liver function marker enzymes (AST, ALT and ALP), varying elevated levels of renal function enzymes (creatinine and uric acid) and depletion of serum proteins when compared with normal control rat (Table 2). Similar elevated levels of AST, ALT and ALP in alloxan-induced diabetic rats have been reported (Gonzalez and Fevery, 1992; Nwanjo, 2008; Akah et al., 2009, Ikewuchi and Ikewuchi, 2012).

Other investigators have similarly reported on higher levels of creatinine (Akah et al., 2009), uric acid (Alarcon-Aguilar et al., 2005) and lower levels of total protein and albumin (Akah et al., 2009) in alloxan-induced diabetic rats when compared with normal control rats. In this study, treatment with *L. africana* leaf powder significantly (p<0.05) reduced the elevated activities of AST, ALT, ALP, and creatinine and marginally (p>0.05) reduced the elevated activity of uric acid in diabetic rats towards the normal control

values. Considerable reduction of the elevated levels of AST, ALT and ALP in the serum of treated diabetic rats suggests that the leaf powder exhibited hepatoproctective effect. Rawi et al. (1998) posited that the decrease of transaminase activities with treatment may be attributed to the improved liver function with return of gluconeogensis towards the normal rate. Creatinine and uric acid are "markers" of kidney function (Newman and Price, 1999). Reduction of both creatinine and uric acid levels in diabetic rats treated with doses of the leaf powder reflects that the test samples exhibited potentials to preserve the renal integrity of the treated rats.

Determination of plasma proteins like albumin can act as a criterion for assessing synthetic capacity of the liver, since nearly all of them are synthesized in hepatocytes (Rasekh et al., 2008). The significant (p<0.05) lower total protein and albumin in the serum of diabetic control rats relative to normal control rats is in agreement with the report by Akah et al. (2009). Decrease in serum proteins tends to reflect chronic liver damage (Rasekh et al., 2008). Sood (2006) had earlier noted that gluconeogensis (protein depletion) is associated with hyperglycaemia and glycosuria.

According to Woodman (1996), the common pattern seen following significant hepatocellular damage is a reduction in albumin accompanied by a relative increase in globulin which leads to albumin/globulin ratio reduction as observed in this study. The restorative effect of the total proteins, albumin, globulin and albumin/globulin ratios in the treated diabetic rats towards the values obtained in the normal control rats clearly demonstrated that the leaf powder exhibited hepatoprotective effect. The observed ameliorative effect of the *L. africana* leaf powder on the biochemical indices in alloxan-induced diabetic rats may be associated with the inherent heterogeneous phytochemicals present naturally in the leaf.

Conclusion

The results of our current study demonstrate that *Lasianthera africana* leaf powder has potentials to ameliorate haematological and biochemical abnormalities associated with alloxan induced diabetes. Further biochemical studies are needed to elucidate the mechanism of action and the active compounds involved in the correction of abnormalities found in the diabetic control rats.

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