Hematological Indices of Diabetic Rats Treated with Crude Extract and Fractions of *Lasianthera Africana* Leaf

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Summary

BACKGROUND

Diabetes adversely affects hematological indices. *Lasianthera africana* (LA) leaf is used in folklore to treat diabetes. Type 2 diabetes can be induced with high fat diet and low dose streptozotocin. The objective of this study was to assess the effects of crude extract and fractions of LA leaf on hematological parameters of male Wistar rats, with induced Type 2 diabetes.

MATERIALS AND METHODS

Crude extract was prepared by macerating the leaf powder in a 50ml methanol and 50ml dichloromethane mixture and concentrating the filtrate in a water bath. Fractions were produced by column chromatography using 50% and 100% methanol. Twenty-five diabetic rats were induced and assigned to groups 2-6. Group 2, diabetic controls (DC) received DMSO; groups 3-6 glibenclamide, crude extract, 50% and 100% leaf fractions, respectively. Group 1 was normal control (NC). Treatment was orally for 28 days and blood collected the following morning for determination of hematological indices using an automated heamanalyzer.

RESULTS

RBC indices (Count, Hb, PCV, MCV, MCH, MCHC) were reduced in diabetic controls (DC) compared to normal controls (NC) but increased in treated diabetic rats compared to DC. Total WBC and Neutrophils were raised while Lymphocytes were reduced in DC compared to NC. Other white cells were insignificantly different in DC compared to NC, and even after treatment with the plant leaf compared to DC. The platelet indices were increased in DC compared with NC but reduced following treatment with the plant leaf and glibenclamide compared to DC.

CONCLUSIONS

Crude extract and fractions of *LA* leaf can reverse hematological anomalies in type 2 diabetic male Wistar rats and may thus be useful in preventing diabetes-induced hematotoxicity.

***Keywords**: Hematological Indices, Lasianthera Africana, Diabetes Mellitus, Crude Extract, Fractions*

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Introduction

Hematopoiesis is a vital physiological process in mammals as it results in the formation of all the cellular components of blood namely, red blood cells (RBCs), white blood cells (WBCs) and platelets. These cells perform life-sustaining functions namely, tissue oxygenation by the RBCs, control of infections/infestations by the WBCs and hemostasis by the platelets. The process of hematopoiesis may be affected by a range of factors including but not limited to exposure to conventional drugs [1], plant extracts [2], environmental pollutants [3], malignancies [4] and acute and chronic diseases [5,6].

Diabetes mellitus (DM) is a chronic endocrine disorder characterized by chronic hyperglycemia from complete or relative lack of insulin secretion or tissue insensitivity to it. The consequence thus manifests in multiple systems. Pathogenesis of DM is linked to the hyperglycemia-induced increase in oxidative stress from enhanced production of Reactive Oxygen Species (ROS) and/or depressed antioxidant mechanisms [7, 8].

The current and projected prevalence of the disease is alarming. Saeed *et al.* [9] reported a global prevalence of 463 million people in 2019, and projected a rise to 578 million and 700 million people by 2030 and 2045, respectively. Type 2 diabetes, the commoner form of the disease [10], is a chronic inflammatory condition [11], and has been induced experimentally with a combination of high fat diet and low dose streptozocin which is mildly toxic to the beta-cells of the pancreas, responsible for production of insulin [12]. Complications of diabetes are many, and can affect virtually any system.

The effect of the disease on the hematopoietic system includes alterations in blood indices [6,13, 14]. The increase in ROS production and non-enzymatic glycation of macromolecules induced by hyperglycemia seem to play a major role. These processes change cellular structure and function, causing the formation of advanced glycation end products (AGEs) which interact with specific receptors for AGEs. The formed complexes enhance metabolic disturbances and further increase ROS production, leading to changes in the structure and biophysical features of vascular endothelium [15]. These structural modifications result in vasodilatation, increased permeability and damage to RBCs with consequent decrease in RBC indices [16]. Hyperglycemia also causes activation of the coagulation cascade thereby contributing to the vascular complications of DM [17]. The disease also promotes differentiation and maturation of WBC [18].

A number of plants exhibit hypoglycemic activity in animal models, and are thus useful in treatment of experimental diabetes. According to Ebong *et al.* [19], some of the plant extracts provide better control of

hyperglycemia than typical oral antidiabetic agents like glibenclamide, and may even reverse some of the complications of the disease.

Lasianthera africana (*Icacinaceae)* is a perennial herb found as under storey in the secondary jungles and thickets of the rain forests of Southern Nigeria and Western Cameroon, extending to Zaire. It is a common plant in Akwa Ibom and Cross River states of Nigeria where its leaves are used as vegetable and in folkloric medicine. The leaf has four ethno-varieties which are identified by their taste, colour and ecological distribution. The biological effects of the plant leaf include antioxidant [20], hypoglycemic [21], antinociceptive, antipyretic [22] antimicrobial [23], anti-ulcerogenic [24], anti-plasmodial [25] and free radical scavenging activities [26]. The leaf has been reported to be safe with an LD_{50} of above 5000 mg/kg body weight [21], and is rich in phenolic compounds [27]. Crude extract and fractions of the plant leaf have been shown to mitigate the deleterious effects of DM on rat testes [28]. The aim of this study was to evaluate the effects of crude extract and fractions of *L. africana* leaf on the hematological parameters of diabetic Wistar rats and also determine whether the leaf can reverse the hematological anomalies induced by DM. To the best of our knowledge, this study is novel in our environment.

Materials and Methods

Preparation of Leaf Crude Extract and Fractions

Fresh leaves of *Lasianthera africana* purchased from a local Market in Calabar South, Cross River State, Nigeria during the rainy season were authenticated in the Department of Botany, University of Calabar, Nigeria. The plant specimen (EUDB S01/41) was preserved in the Departmental herbarium for future reference. The leaves were cleaned, dried on the laboratory work top surface then pulverized into powder using a manually operated grain mill. Five hundred milligrams of the leaf powder was soaked in a solvent (mixture of 50 ml methanol and 50 ml dichloromethane) and allowed to stand for 48 hrs before filtering with Whatman filter paper (No.1). The filtrate was concentrated in a thermo- regulated water bath ($44\text{-}46^0$C). The pasty concentrate formed the crude extract which was stored in a refrigerator until required for the work.

The fractions were prepared as earlier documented [28]. Ten milligrams of the prepared pasty crude extract was dissolved separately in 50 percent and 100 percent methanol. Each solution was subjected to column chromatography, and the eluent was concentrated in a thermo-regulated water bath to form either a 50% or 100% methanol fraction stock. This stock was again stored in a refrigerator until needed for the study. All the leaf preparations were dissolved in dimethylsulphoxide (DMSO) as a vehicle, for administration to the rats.

Experimental Animals

Mature male Wistar rats weighing 180-200 grams, purchased from the animal house, Department of Pharmacology, University of Calabar, were used for the study. Animals were kept in plastic cages, for seven days to acclimatize to the laboratory environment and maintained in the same condition throughout the study period. They were fed with standard rat food pellets and allowed clean water *ad libitum.* Handling of animals was in conformity with the International Guidelines for the Care and Handling of Experimental Animals [29], and the study protocol was duly obtained from the Faculty of Basic Medical Sciences Research and Ethical committee, University of Calabar, Nigeria.

Acute Toxicity Study

Oral acute toxicity test was performed using the Organization of Economic Cooperation and Development (OECD) guideline for testing of chemicals 423 [30].

Induction of Experimental Diabetes Mellitus

Experimental DM was induced as earlier described [31] using a high fat-diet (HFD) and low dose streptozotocin (STZ). High fat diet was prepared as earlier described [32], and liberally given to male rats for 21 days. On the morning of the 22nd day after an overnight fast, a low dose of STZ (30mg/kg body weight) dissolved in 0.1 M sodium citrate buffer (pH 4.7) was injected intra-peritoneally. Forty-eight hours post injection; the fasting blood glucose levels of the rats were determined with a glucometer using blood from the tail tips of the rats. Animals with fasting blood glucose level above 200mg/dl were considered diabetic [33], and used for the study.

Treatment Groups

The diabetic rats were randomly divided into five groups (n=6) labelled 2-6. Group 2 (diabetic controls) received the vehicle (DMSO); group 3 rats were treated with glibenclamide (5mg/kg); groups 4, 5, and 6 were administered crude ethanol leaf extract (400mg/kg), 30% leaf fraction (400mg/kg) and 100% leaf fraction (400mg/kg), respectively. Group 1 (Normal control) rats were non- diabetic. Administrations were daily through an oral cannula for 28 days. At the end of the last administration, the animals were fasted overnight, anaesthetized the next morning with ether vapour and blood collected through cardiac puncture into ethylene diamine tetra-acetate (EDTA) containing bottles.

Hematological Assessment

The blood so collected was used to assess hematologic indices of the rats using Automated Haemanalyser (Bedfordshire, UK) calibrated according to the manufacturer's instructions for analysis of human blood, and accurately programmed for analysis of RBC count, hemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), WBC count and differentials, platelet count, plateletcrit (PCT), mean platelet volume (MPV) and platelet distribution width (PDW).

Statistical Analysis

Results were analyzed using one-way analysis of variance (ANOVA) followed by Student t-test and presented as mean ± standard error (SE). *P* value of less than 0.05 was considered statistically significant.

Results

Acute Toxicity Test

Acute toxicity studies carried out on the crude extract and fractions of *Lasianthera africana* leaf did not yield any lethality or visible signs of toxicity (e.g., salivation, sedation, paw-licking, writhing, change in body weight) in the rats up to a dose of 5000 mg/kg after 24 hours. Further monitoring for 7 days did not change its toxic state. Hence, an LD_{50} of more than 5000 mg/kg body weight was adopted. The dose of the different leaf preparations used for the study was 8 percent of its LD_{50} - 400 mg/kg body weight.

Effect of Treatments on Red Blood Cell Indices in Male Wistar Rats

Findings are presented in Table 1 presented in the appendix. Diabetic control rats had significantly reduced ($P<0.05$) RBC counts, hemoglobin, packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) compared to normal control rats. Treatment of diabetic rats with crude extract and fractions of *Lasianthera africana* leaf caused marked rise in the mentioned parameters, and the values were comparable with that of the standard drug, glibenclamide (Table 1).

Effects of Treatment on White Blood Cell Count and Differentials in Male Wistar Rats

Table 2 summarizes the observed changes in WBC indices with the different treatments. WBC count and percentage of neutrophils were raised significantly ($P<0.05$) in diabetic rats compared to normal control while lymphocyte count was reduced. Following treatment with crude extract and fractions of the plant leaf, the total white cell count and neutrophil percentages were reduced significantly ($P<0.05$) while the lymphocytes percentage was increased. There were insignificant ($P>0.05$) changes in the values for monocytes, eosinophils and basophils in diabetic rats compared to normal controls, and even after different treatments of the diabetic rats, the changes remained insignificant compared with the diabetic control.

Effects of Treatment on Male Rat Platelets Indices (Count, PCT, MPV and PDW)

Platelet indices were higher in diabetic rats compared to normal control, but were reduced upon treatment with both the plant leaf preparations and glibenclamide (Table 3).

Discussion

Hematological parameters give information on the physiological states of blood cells, and these parameters may suffer distortions in type 2 Diabetes Mellitus. In this study, the indices (RBC, WBC and platelet indices) were altered in diabetic control rats as compared to normal rats, a finding that is in consonance with findings by earlier researchers [13, 14, 6].

Red blood cell indices are used to evaluate erythropoiesis. Alterations in the process may be in the direction of a decrease, indicated by anaemia or an increase, indicated by polycythemia. Findings of reduced RBC indices (count, Hb, PCV, MCV, MCH and MCHC) in diabetic control rats compared to the normal group thus suggests anaemia, consistent with previous reports [13, 33].

The reductions probably resulted from damage to RBCs from structural alterations to their membranes brought about by the excess ROS generated in DM [34]. In addition, glycosylation of hemoglobin known to occur in diabetic states [35] may contribute to the low Hb concentration. The observed increase red cell indices in diabetic rats following treatment with the plant leaf also suggests enhanced erythropoiesis. Some of the chemical constituents of the leaf may be responsible for the observation.

For example, flavonoids stimulate the production of erythropoietin, the key hormonal stimulant of erythropoiesis, and prevent free radicals-induced hemolysis of RBCs [36, 37]. Also, the oxidative stress linked with the disease may have been attenuated by the leaf because of its antioxidant action [20], and free radical scavenging activity [26]. The improved levels of MCV, MCH and MCHC may be linked to an increase in erythropoiesis.

Elevated WBC count is a classical marker of inflammation and is associated with type 2 diabetes mellitus as well as other diseases [38]. The increase in the diabetic state is due to hyperglycemia-induced oxidative stress, advanced glycation end products and the activities of cytokines and angiotensin 11 [18]. The observed significant ($P<0.05$) rise in WBC count in diabetic control rats compared to normal controls is consistent with earlier reports [11, 18, 38].

The reduction in WBC count upon treatment of diabetic rats with *Lasianthera africana* leaf may be attributable to its anti-inflammatory action [22], antioxidant effect [20] and free radical scavenging activity [26]. The other effects of chronic hyperglycemia mentioned above may have been countered by the hypoglycemic effect of the leaf [21]. The study also showed decreased lymphocytes and increased neutrophils in the diabetic control group when compared with normal rats.

The decrease in lymphocytes may be a response to the stress associated with the disease since they are essential for maintenance of the body's defense /immunity [39]. The increase in neutrophils may be due to involvement of these cells in the phagocytic processes against different antigens especially as the risk of infection is higher in diabetics [40]. The result relates with earlier reports [33, 41].

Platelets play a major role in hemostasis, wound healing, angiogenesis as well as pathogenesis of many inflammatory diseases, and its indices are indicators of diabetic microvascular complications [42]. Our study yielded higher platelet indices for diabetic controls compared to normal rats. The significant ($P<0.05$) increase in count is in accord with earlier findings [17, 42, 43], and may have resulted from activation of the megakaryocyte-platelet system which occurs in diabetes resulting in increased turnover of platelets [44].

The observed increase in plateletcrit (PCT) is naturally explainable by the raised platelet count since it represents the percentage of blood volume occupied by platelets and is calculated from the formula, platelet count × MPV / 10,000 [45]. It has been shown that increased MPV depicts larger platelet diameters, which can be used as a marker of platelet production and activation rates [46]. It can thus be opined that the significant rise of MPV in diabetic control rats compared with the normal control group results from increased platelet turnover and activation which are common features of DM as earlier mentioned.

The rise in the Platelet Distribution Width (PDW) is still linkable to the activated megakaryocyte-platelet system because of platelet anisocytosis resulting from the process [47]. Treatment of diabetic rats with different preparations of the plant leaf yielded reduced platelet indices compared to DC; implying that the effects of DM-induced activation of the megakaryocyte- platelet pathway was attenuated.

Since hyperglycemia is behind the activation [17], the leaf extract may have produced the observed effects through reduction in blood glucose since it has hypoglycemic activity [21].

Probably, the plant leaf extract also produced the observed effect through its reported antioxidant and anti-inflammatory actions earlier stated. The fact that the values of platelet indices in leaf-treated rats were not significantly different from those treated with glibenclamide, a known oral hypoglycemic agent further gives credence to the above proposition.

The values of median lethal dose (LD_{50}) have been used to classify the toxicity of test compounds, with values of 5000-15,000 mg/kg being considered practically non-toxic [50]. Thus, since the crude extract and fractions of *L. Africana* leaf gave an LD50 of more than 5000 mg/kg, the plant leaf might be regarded as non-toxic and safe in this model. This finding correlates with an earlier report [21].

Conclusion

Findings suggest that the crude extract and fractions of *Lasianthera africana* leaf can reverse the hematological anomalies associated with diabetes-induced with HFD/ STZ in male Wistar rats. The effects were evidenced by improved RBC, WBC and platelet indices; dose dependently more marked in the leaf fractions group, and comparable with those of glibenclamide. The plant leaf extract may be a useful agent for prevention of diabetes-induced hematotoxicity especially as it is safe in the studied model.

Authors' Contribution

GAE conceived the idea and gave the study design. All the authors were involved in the experimentation. WOE and AIU analyzed and interpreted the obtained data while ESU and MUA wrote the first draft. KFO critically revised the manuscript for important intellectual content, and the final approved version submitted was by GAE.

Conflict of Interest

We declare that there was no conflict of interests.

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Appendix

Table: 1 Effect of Treatments on Red Blood Cell Indices in Male Wistar Rats.

Parameters	Study groups: Normal control	Diabetic control	Diabetic + gilbencl.	Diabetic + 30% fctn	Diabetic + 100% fctn	Diabetic + crude ext.
RBC (x 10^6/µl)	8.94±0.43[a]	4.52±0.13[b]	8.28±0.38[a]	8.76±0.09[a]	8.15±0.13[a]	8.83±0.05[a]
HB (g/dl)	15.78±0.66[a]	8.43±0.30[b]	15.31±0.58[a]	15.17±0.18[a]	15.53±0.51[a]	15.88±0.24[a]
PCV (%)	46.83±2.55[a]	25.47±1.21[b]	49.42±2.12[a]	47.88±0.83[a]	50.07±0.53[a]	48.60±1.18[a]
MCV (fl)	54.89±2.11[a]	32.76±1.14[b]	54.13±0.91[a]	53.45±1.11[a]	53.05±1.07[a]	53.69±1.27[a]
MCH (p_g)	17.38±0.69[a]	13.50±0.29[b]	17.08±0.69[a]	16.93±0.28[a]	16.45±0.58[a]	17.21±0.16[a]
MCHC (g/dl)	31.67±0.52[a]	28.68±0.31[b]	31.04±0.19[a]	30.74±0.38[a]	31.00±0.74[a]	32.26±0.80[a]

Values are means±SE. n = 6.
Means with same superscript along the longitudinal axis are not significantly different.
gilbencl. = glibenclamide, Fctn and ext.= Fraction and extract of *Lasianthera africana* leaf respectively,
RBC = red blood cell, HB = hemoglobin, PCV = packed cell volume, MCV = mean corpuscular volume,
MCH = mean corpuscular hemoglobin, MCHC = mean corpuscular hemoglobin concentration.

Table 2: Effect of Treatment on White Blood Cell Counts and Differentials in Male Wistar Rats

Parameters	Study groups: Normal control	Diabetic control	Diabetic + gilbencl.	Diabetic + 30% fctn	Diabetic + 100% fctn	Diabetic + crude ext.
WBC (x10^3/ µl)	7.42±0.68[a]	11.32±1.03[b]	7.75±0.75[a]	7.58±2.10[a]	7.28±1.07[a]	8.7±1.49[a]
Neutrophils (%)	44.09±1.85[a]	55.03±1.22[b]	43.56±1.51[a]	45.48±2.01[a]	44.53±1.93[a]	45.00±2.63[a]
Lymphocytes (%)	49.50±0.78[a]	38.50±0.22[b]	50.01±0.39[a]	48.13±1.74[a]	49.10±2.00[a]	48.60±2.63[a]
Monocytes %	4.11±0.11[a]	4.20±0.35[a]	4.19±0.37[a]	4.13±0.55[a]	4.13±0.55[a]	4.09±0.55[a]
Esinophils %	2.09±0.50[a]	2.01±0.64[a]	2.02±0.71[a]	2.06±0.63[a]	2.04±0.70[a]	2.10±0.62[a]
Basophils %	0.21±0.14[a]	0.26±0.07[a]	0.22±0.10[a]	0.20±0.16[a]	0.20±0.15[a]	0.21±0.18[a]

Values are means ±SEM n = 6
Means with same superscript along the longitudinal axis are not significantly different.
Gilbencl. = glibenclamide.
Fctn and ext.= Fraction and extract of *Lasianthera africana* leaf respectively.
WBC = white blood cell

Table 3: Effects of Treatment on Male Rat Platelets Indices (Counts, PCR, MPV and PDW).

Parameters	Study groups Normal control	Diabetic control	Diabetic + gilbencl.	Diabetic + 30% fctn	Diabetic + 100% fctn	Diabetic + crude ext.
PLT ($x10^3$/ µl)	215.23±46.49[a]	351.32±31.03[b]	247.75±41.75[a]	255.58±29.10[a]	232.28±21.07[a]	268.70±28.49[a]
PCT (%)	0.23±0.02[a]	0.28±0.02[b]	0.23±0.01[a]	0.24±0.01[a]	0.23±0.01[a]	0.24±0.01[a]
MPV (fL)	6.74±0.38[a]	7.98±0.25[b]	7.01±0.39[a]	7.13±0.34[a]	6.96±0.42[a]	7.15±0.33[a]
PDW (fL)	13.88±0.56[a]	15.91±0.77[b]	14.11±0.33[a]	14.31±0.24[a]	14.03±0.41[a]	14.26±0.

Values are means ±SEM n = 6
Means with same superscript along the longitudinal axis are not significantly (P>0.05) different.
Gilbencl. = glibenclamide. Fctn and ext.= Fraction and extract of *Lasianthera africana* leaf respectively.
PLT = Platelets, PCT = Plateletcrit, MPV = Mean platelet volume, PDW = platelet distribution width