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RESEARCH ARTICLE

Anti-Diabetic Potential of Methanolic Leaf Extract of *Kigelia africana* (Lam.) Benth in Alloxan-Induced Diabetic Rats

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ABSTRACT

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia and disturbances of carbohydrate, fat, and protein metabolism associated with complete or relative deficiency in insulin secretion or insulin activity. The aim of this research is to investigate the anti-diabetic activities of methanol leaf extract of *Kigelia africana* on alloxan-induced diabetic rats. The leaf extract was screened for anti-diabetic potential. Twenty rats 3–4 weeks old of mixed sex weighing 60–100 g body weight were divided into five groups (GI-GV), each group containing four rats, Groups GII–GV were induced with diabetes, while GI served as normal control, GII which is the diabetic control is fed with only feed and distilled water, GIII was administered with 5 mg/kg bw of standard anti-diabetic drugs Glibenclamide (GLB), while GIV was orally administered 250 mg/kg bw of leaf extract of *K. africana* (LEKa) and GV was orally administered 500 mg/kg bw of LEKa. After 14 days of oral administration of extract, the animals were sacrificed and the serum collected for analysis of liver and kidney parameters, lipid profile (total cholesterol, low density lipoprotein cholesterol, high density lipoprotein cholesterol, and triglyceride), hematological parameters (pack cell volume, hemoglobin, white blood cells, lymphocytes, and neutrophils). The LD_{50} by oral route in rats was >2000 mg/kg body weight. In conclusion, LEKa exhibited anti-diabetic potential in alloxan-induced diabetic rats.

Keywords: Alloxan, diabetes mellitus, kidney, *Kigelia africana,* liver

INTRODUCTION

The *Kigelia* plants have a long history of use by rural communities, especially for its medicinal properties. These properties are found in all parts of the tree, including fruit, bark, roots, and leaves, which are used for medical purposes. In conventional medical practice, the present therapies of diabetes mellitus are reported to be unaffordable or unavailable and mostly having unwanted side effects, especially in poor resource economics.[1] Herbal medicines are affordable, easily accessible, and are firmly embedded within wider belief systems of many people in the underdeveloped and developing countries [Figure 1].

Liver is a discrete largest organ in human body that has many interrelated functions and it may be damaged due to one or more of the following: injury from metabolic disturbances, injury from toxins, drugs, chemicals and poisons, lesion of biliary tract, certain viral infections, hypoxia, and tumors.[2] Increased activities of liver enzymes such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), and γ-glutamyl transpeptidase, in the serum/plasma, are indicators of hepatocellular injury. Increased activity of these markers is associated with insulin resistance, metabolic syndrome, and type 2 diabetes. An association exists between diabetes and liver injury.[3,4] The kidneys excrete metabolic waste products and the serum concentrations are regulated from variety of substances. At certain level of renal failure, the following routinely

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Figure 1: *Kigeliaafricana* leaf

measured substances often become abnormal and the extent of the abnormality generally depends on the severity of the disease. Serum creatinine, urea, and electrolyte concentrations change inversely with changes in chronic renal failure.

METHODOLOGY

Collection and Identification of the Plant

The leaf of the plantas collected from villages around Dutsin-Ma Local Government, Katsina state, Nigeria. Botanical identification was done at Botany unit of Federal University Dutsin-Ma and voucher number was assigned to the specimen which was deposited in the herbarium of the same institution for reference.

Preparation of the Methanolic Leaf Extract

The extraction procedure was carried out according to.[5] The leaves were dried at room temperature in the Biochemistry Laboratory, the dried leaves were pounded using mortal and pistil and, then, sieved to powder using a sieve. 200 g of the powdered sample was dissolved in 1000 mL of methanol and allowed to stay for 72 h with periodic stirring. The sample was filtered using Whatman number 1 filter paper, the filtrate was then placed in the ovum at 80°C and complete drying took 8 h. The extraction was repeated using 200 g of the powered part, in other to have more extracts.

Experimental Animal

Twenty (20) rats weighing 60–100 g mixed sex were purchased from Department of Veterinary Medicine ABU Zaria, Kaduna State, Nigeria. The rats were allowed to acclimatize for 2 weeks. The rats were fed with starter mesh with unlimited access to distilled water. They were kept in well-ventilated cages at the animal facility in Federal University Dutsin-Ma, Katsina State.

Animal Induction

The induction of diabetes with alloxan was done intraperitoneally with 100 mg/kg body weight of the alloxan and diabetes was confirmed after 72 h.

Experimental Design

Twenty (20) Wister albino male rats were assigned into five different groups which had four rats in each of the groups.

Group I: Non-diabetic and no treatment (normal control).

- Group II: Diabetes induced rats without treatment (negative control).
- Group III: Diabetes induced rats treated with standard drug (GLB 5 mg/kg).
- Group IV: Diabetes induced rats treated with methanol leaf extracts of *Kigelia africana* (250 mg/kg b.w).
- Group V: Diabetes induced rats treated with methanol leaf extracts of *K. africana* (500 mg/kg b.w).

The treatment was done orally for a period of 14 days (daily).

Acute Toxicity Studies

The observation and monitoring of the parameters commenced immediately after administering the extract. The rats were kept under the same conditions and observed for a total of 7 days, for signs of toxicity, which include paw-licking, motor activity, tremors, convulsions, posture, spasticity, opisthotonicity, ataxia, sensations, piloerection, ptosis, lacrimation, exophthalmos, salivation, diarrhea, writhing, skin color, respiratory rate, and mortality.[6]

A leaf extracts of *K. africana* (50, 500, 1000, and 2000 mg kg^{-1} b wt.) were administered to 1–4 groups

Weight and dosage for toxicity test

Groups	Weight (Kg)	Dosage (mg/kg)	Days of observations
1.	90–110	50	7
2.	90–110	500	7
3.	90–110	1000	7
4.	90–110	2000	7

of two rats each in a single oral dose using feeding needle. The toxicological effect was assessed on the basis of mortality, which was expressed as LD_{50} and was calculated using the limit test dose, up and down procedure of Organization for Economic and Cultural Development.[7]

Estimation of Glucose Levels

Serum glucose was estimated by glucose oxidase method using Randox kit.[8]

Estimation of Serum Lipid Profile

Serum lipid profile was enzymatically determined using Randox kit and the test was based on the following methods: Serum total cholesterol (TC),[9] high-density lipoprotein cholesterol (HDL-C),[10] and triglycerides (TG).[11] LDL-C and VLDL-C were calculated using Friedewald's formula;[12]

- LDL-C (mmol/L) = TC - (HDL-C)-TG/2.2
- VLDL-C (mmol/L) = TG/2.2

Determination of Serum Creatinine

Serum creatinine was determined by enzymatic method.[13]

Determination of Serum Electrolyte

Serum sodium and potassium were estimated by flame photometer.[14] Serum chloride and bicarbonate were determined by the method.[15]

Determination of Serum Urea

Serum urea was determined by enzymatic method.[16,17]

Determination of Liver Function Biomarkers

Plasma enzymes such as AST, alkaline phosphatase (ALP), and ALT were determined using Randox diagnostic kits. Thetotal protein and total bilirubin (BIL) were also determined using Randox diagnostic kits.[18]

Statistical Analysis

The experimental results obtained are expressed as mean ± standard deviation (SD). The data were subjected to one-way analysis of variance (ANOVA) and differences between samples were determined by Tukey multiple comparison tests using the SPSS 16.0 (statistical program for social sciences). The level of significance was set at $P < 0.05$.

RESULTS

Acute Toxicity

Oral administration of methanolic leaf extracts of *K. africana* (2000 mg/kg body weight) did not caused mortality, but few clinical signs of toxicity in rat in the first 24 h and during the 7 days observation period. The animals show some behavioral changes such as excitement, slow movement, weight reduction, and loss of appetite. Therefore, the medium lethal dose (LD_{50}) is >2000 mg/kg [Table 1].

Serum Glucose Level

Table 2 shows the effect of *K. africana* extracts on fasting blood glucose (FBG) level which were measured on 3rd, 6th, 9th, 12th, and 15th day of post-induction and compared with normal and diabetic control groups. The values in the table below of Alloxan-induced rats showed a significant increase ($P < 0.05$) in the FBG level compared to normal rats. Oral administration of GLB 5 mg/kg methanolic LEKa at the dose of 250 mg/kg and 500 mg/kg body weight showed a significant decrease in FBG level on the 6th 9th 12th, and 15th day when compared with the diabetic control group.

Result of Serum Lipid Profile

Table 3 showed significant increased ($P < 0.05$) in the serum level of TC, TG, and LDL cholesterol, while HDL shows a significant decrease ($P < 0.05$) compared to normal control. The standard drug treated group (GIII) shows a significant decrease ($P < 0.05$) in total cholesterol TG and LDL when compared with diabetic control group. The extracts treated group shows a decrease in serum level of TC, TG, and LDL cholesterol when compared with diabetic control group. The extract treated group (GV) showed significant increase in serum level of HDL-cholesterol.

Result of Hematological Parameters

There were decreased in all parameters across the table in the diabetic untreated group (GII) compared with the normal control. WBC, Hb, and Neutrophiles showed a significant increased ($P < 0.05$) in group (GV) treated with 500 mg/kg body weight compared to the diabetic control group [Table 4].

Result of Liver Function Indices

Table 5 showed significant increased ($P<0.05$) in TB, CB, SGPT, and TP, while SGOT and ALP show a significant decrease ($P<0.05$) compared to normal control. The standard drug treated group (GIII) shows a significant decrease ($P<0.05$) in T.CHOL TG and LDL when compared with diabetic control group. The extracts treated group shows a decrease in serum level of SGPT, and ALP with significant increase in TB, SGOT, ALB, and TP when compared with diabetic control group. The extract treated group (GIV and GV) showed significant decrease in TB, CB, SGPT, and TP with increase in SGOT, ALP, and ALB.

Result of Kidney Function Indices

Table 6 shows significant decrease ($P < 0.05$) in K^-, Cl^- and increase in HCO_3^-, creatinine with normal levels of Na+, and urea compared to normal control. The standard drug-treated group (GIII) shows a significant decrease ($P < 0.05$) in the levels of HCO_3^-, creatinine and increase levels of Na^+ K^-, Cl^-, and urea when compared with diabetic control group. The extracts treated group shows an increase in serum level of Na^+ K^-, Cl^- and urea when compared with diabetic control group.

DISCUSSION

Acute toxicity studies of the leaf extract reveal some behavioral changes such as excitement, slow movement, weight reduction, and loss of appetite when 2000 mg/kg body weight of *K. africana* leaf extracts were administered. No animal died, and thus, the LD_{50} obtained was greater than 2000 mg/kg. The blood glucose levels significantly return to normal after two weeks administration of LEKa in diabetic rats [Table 2]. The treatment group (GIV

Table 1: Acute toxicity test of leaves extract of *Kigelia africana*

Method	Limit test dose	Observation period	Sign of toxicity	Mortality	Ld 50 value
OECD 423 guidelines (2001)	2000 mg/kg	7 days	Nil	0	>2000 mg/kg

Table 2: Effect of leaf extract of Kigelia africana on blood glucose level of alloxan induced diabetic rats

Groups Treatment	Before Induction	48 h after induction	3rd day Induction and extract administered	6th day Induction and extract administered	9th day Induction and extract administered	12th day Induction and extract administered	15th day Induction and extract administered
GI NC	7.67±0.06^a	7.80±0.04^a	8.02±0.07^a	8.35±0.11^a	5.53±0.48^a	7.85±0.30^a	7.92±0.29^a
GII DC	6.85±0.30^b	13.20±0.30^b	13.70±0.37^b	14.20±0.21^d	14.77±0.85^b	15.47±0.67^c	15.83±0.28^b
GIII GLB	9.17±0.49^c	12.15±0.47^c	11.82±1.24^c	11.63±0.53^c	7.25±1.15^c	8.98±0.21^b	7.18±0.42^a
GIV LEKa 250mg/kg bw	6.57±0.11^b	11.32±0.72^d	10.95±0.29^{bc}	10.95±0.75^b	7.38±1.06^c	7.45±0.79^a	7.00±0.86^a
GV LEKa 500mg/kg bw	7.00±0.30^a	11.07±0.71^d	8.52±1.47^a	9.20±1.01^{ab}	7.05±0.25^c	6.17±0.14^d	5.97±0.69^c

Values with different alphabetical superscript along a column are significantly different at $P<0.05$, $n=4$. LEKa: Leave extract of *Kigelia africana*, GLB: Glibenclamide, NC: Normal control, DC: Diabetic control

and GV) showed significant ($P < 0.05$) reduction in blood glucose levels. The group IV and V extract treated group indicated a similar effect with the standard drug. Alloxan induces diabetes mellitus, by partially damaging the beta cells of the pancreas which cause increase in blood glucose concentration observed in rats.[19] A study reported that Stem Bark Extract of *K. africana* reduced blood sugar levels.[19] The diabetic control rats had elevated mean TC, TG, and low density lipoprotein (LDL) [Table 3]. LEKa had shown a hypolipidemic effect by significantly reducing ($P < 0.05$) TC, TG, LDL, and increasing the level of HDL compared to the diabetic control group with the highest reduction noticed in GV administered 500 mg/kg extract. The cholesterol, TG, and LDL lowering effect coupled with HDL elevating effect of the extract have been suggested to be helpful in reducing complications associated with hyperlipidemia as a result of diabetes mellitus.[19]

Table 3: The effect of *Kigelia africana* leaf extract on the lipid profile of alloxan-induced diabetic rats

Groups Treatment	Total cholesterol mMol/L	HDL-C mMol/L	TG mMol/L	LDL-C mMol/L
GI NC	3.13±0.15^{a}	0.50±0.05	0.96±0.03^{a}	2.18±0.11^{a}
GII DC	4.03±0.33^{b}	0.20±0.60	1.43±0.14^{b}	2.96±0.03^{a}
GIII GLB 5 mg/kg B.W	3.17±0.09^{a}	0.75±0.06^{c}	0.95±0.06^{a}	1.99±0.04^{b}
GIV LEKa 250 mg/kg B.W	3.50±0.06^{a}	0.73±0.05^{c}	1.15±0.10^{b}	2.26±0.08^{a}
GV LEKa 500 mg/kg B.W	3.63±0.12^{a}	1.06±0.06^{c}	1.30±0.10^{b}	1.99±0.06^{b}

Values with different alphabetical superscript along a column are significantly different at $P<0.05$, $n=4$. LEKa: Leave extract of *Kigelia africana*, GLB: Glibenclamide, NC: Normal control, DC: Diabetic control

Table 4: Effect of leaf extract of *Kigelia africana* on PCV, Hb, WBC, Neutrophiles, and Lymphocytes of induced diabetic rats.

Groups Treatment	PCV	Hb	WBC	NEUT	LYMPH
GI NC	40.33±0.33^{a}	13.27±0.03^{a}	2.73±0.18^{a}	25.00±3.60^{a}	73.33±3.53^{a}
GII DC	37.33±1.20^{b}	13.80±0.42^{a}	2.50±0.21^{a}	23.67±1.86^{a}	64.67±2.60^{b}
GIII GLB 5 mg/kg B.W	41.00±0.91^{a}	13.68±0.32^{a}	8.05±1.71	27.00±4.38^{a}	72.75±4.40^{a}
GIV LEKa 250 mg/kg B.W	42.00±1.73^{a}	14.13±0.48	4.05±0.54	33.50±3.52	66.50±3.52^{b}
GV LEKa 500 mg/kg B.W	41.00±0.58^{a}	13.63±0.24^{a}	10.83±1.41	41.33±6.69	58.67±6.69

Values with different alphabetical superscript along a column are significantly different at $P<0.05$, $n=4$. LEKa: Leaf extract of *Kigelia africana*, GLB: Glibenclamide, NC: Normal control, DC: Diabetic control

Table 5: Effect of *Kigelia africana* leaf extract on liver function indices of alloxan induced diabetic rats.

Groups Treatment	T.B	CB	SGOT	SGPT	ALP	ALB	T.P
GI NC	1.83±0.23^{a}	0.76±0.33^{a}	9.33±0.66^{a}	10.00±1.15^{a}	96.6±13.67^{a}	4.00±0.11^{a}	6.10±0.05^{a}
GII DC	2.13±0.08^{b}	1.10±0.25^{b}	8.66±0.66^{b}	12.00±0.00^{b}	86.00±3.46^{b}	3.90±0.25^{b}	6.33±0.33^{a}
GIII GLB	2.42±0.34^{b}	1.00±0.15^{b}	10.00±0.81^{c}	9.00±1.00^{c}	85.50±2.98^{b}	5.25±1.20^{c}	6.40±0.18^{a}
GIV LEKa 250mg/kg bw	1.75±0.25^{a}	0.70±0.07^{a}	9.50±0.50^{a}	9.00±0.57^{c}	98.75±3.54^{a}	4.80±0.25^{a}	6.17±0.34^{a}
GV LEKa 500mg/kg bw	1.83±0.21^{a}	0.76±0.03^{a}	10.00±1.15^{c}	10.00±0.00^{a}	100.33±1.85^{c}	4.20±0.11^{a}	5.50±0.37^{b}

Values are mean±SD; $n=5$; NC: Normal control DC: Diabetic control GLB: Glibenclamide, LEKa: Leave extract of *Kigelia Africana*, T.B: Total bilirubin CB: Conjugate bilirubin, SGOT: Serum glutamic oxaloacetate transaminase SGPT: Serum glutamate pyruvate transaminase

Table 6: Effect of *Kigelia africana* leave extract on kidney function of experimental diabetic rats

Groups Treatment	Na^{+}	K^{-}	Cl^{-}	HCO^{3-}	Urea	Creatinine
GI NC	138.66±1.20^{a}	4.00±0.11^{a}	97.66±2.33^{a}	25.00±1.52^{a}	9.60±0.26^{a}	88.00±7.02^{a}
GII DC	138.00±1.52^{a}	3.23±0.88^{b}	96.00±1.15^{b}	25.00±0.88^{a}	9.56±0.44^{a}	89.33±3.52^{a}
GIII GLB 5mg/kg	139.75±0.94^{b}	3.92±0.20^{b}	99.50±2.21^{ab}	25.66±0.86^{a}	10.67±0.33^{b}	76.50±3.57^{c}
GIV LEKa 250mg/kg bw	139.00±1.00^{b}	3.85±0.06^{b}	102.50±1.50^{c}	25.00±0.91^{a}	9.17±0.34^{a}	86.00±4.81^{ab}
GV LEKa 500mg/kgbw	139.33±1.33^{b}	3.86±0.20^{b}	101.33±1.76^{c}	24.00±0.00^{b}	11.46±0.29^{c}	87.66±2.02^{ab}

Values are mean±SD; $n=4$. NC: Normal control DC: Diabetic control GLB: Glibenclamide, LEKa: Leave extract of *Kigelia Africana*, Na: Sodium, K: Potassium Cl: Chloride, HCO_3: Bicarbonate, U: Urea, Cr: Creatinine

The administration of LEKa led to a significant increase in all the hematological parameters studied. The diabetic rats orally administered 250 mg/kg b.w and 500 mg/kg b.w with significant change ($P > 0.05$) in hematological parameters when compared to normal control [Table 4]. Hematological parameters evaluation has been reported to be a useful tool in revealing the harmful effect of plant extracts on animal's blood composition and determining possible alterations in enzyme activities due to tissue damage (e.g., liver) as documented by.[20] The existence of anemia as one of the complications in diabetes mellitus might be linked to an upsurge in non-enzymatic glycosylation.[21]

In Table 5, the extract-treated group (GIV and GV) showed significant reductions of TB, CB, SGOT, and TP and increased in the level of SGPT, ALP, and ALB compared to diabetic untreated group. The renal parameters [Table 6] had significantly ($P < 0.05$) elevated levels of creatinine, with normal levels of sodium ion, bicarbonate, urea, and with significantly reduced ($P < 0.05$) levels of chloride and potassium ion compared to normal control group. The significant ($P < 0.05$) raised activities of ALT and AST may have resulted from possible necrotic injury of the liver and cholestasis.[22] ALP is a marker enzyme for the plasma membrane and endoplasmic reticulum. ALP is typically employed in the assessment of functional integrity of the plasma membrane.[23]

CONCLUSION

The result of the study indicated the anti-diabetic effect of the administration of methanolic leaf extract of *K. africana* in experimental rats. The extract was safe at low doses. There were no deaths or signs of toxicity in treated rats during the 7 days toxicity study, it is possible to suggest that the LD_{50} of *K. africana* is greater than 2000 mg/kg body weight. Thus, its continued usage as traditional medicine is recommended.

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