UMYU Scientifica, Vol. 1 NO. 2, December 2022, Pp 90 – 97
https://doi.org/10.56919/usci.1222.011
ISSN: 2955 – 1145 (print); 2955 – 1153 (online)
A periodical of the Faculty of Natural and Applied Sciences, UMYU, Katsina

ORIGINAL RESEARCH ARTICLE

Acute Toxicity Studies and Anti-diabetic Activity of Methanolic Root Extract of *Kigelia africana* (lam.) Benth in Alloxan Induced Diabetic Rats.

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ABSTRACT
Diabetes mellitus (DM) is a metabolic disease which results in elevated blood sugar levels. Kigelia africana plant has many medicinal properties due to the presence of numerous secondary metabolites. The aim of this research was to evaluate the acute toxicity profile and anti-hyperglycemic potential of methanol root extract of Kigelia africana on alloxan-induced diabetic rats. Twenty (20) 3-4 weeks old albino rats of mixed sexes with body weight 60-100 g were grouped into five groups (GI - GV) of equal number of rats. Diabetes was induced in the rats of Groups GII -GV, while GI rats were considered as non-diabetic control. GII which is the Diabetic control were received no treatment whereas, GIII were administered orally with 5 mg/kg b.w of standard anti-diabetic drugs Glibenclamide, GIV were orally administered 250 mg/kg b.w of extract and GV were orally administered 500 mg/kg b.w of extract. After 14 days of the treatments, the animals were sacrificed and their serum was analysed for lipid profile, hematological parameters, liver and kidney indices. At the end of the study, the root extract was found to posses' anti-diabetic potential and due to less toxicity at low dose of about 500 mg/kg, the extract is believed to be safe for consumption at low dosage.

ARTICLE HISTORY
Received August 14, 2022
Accepted September 29, 2022
Published December 30, 2022

KEYWORDS
Kigelia africana, anti-diabetic, acute toxicity, Alloxan, Albino rats

INTRODUCTION
Herbal medicine has now been a greater alternative to orthodox medicine in recent times, leading to a subsequent increase in herbal medicine preparations (Sushruta *et al.,* 2007; Ogbonnia *et al.,* 2016). Different parts of African indigenous plants with potential medicinal properties like *K. africana* (Lam.) Benth and *Sorghum bicolor* (L.) Moench have been used by herbal medicine practitioners in the treatment of various diseases in Nigeria (Gupta and Jain, 2019). *K. africana* has many medicinal properties due to the presence of numerous secondary metabolites (Obianagha *et al.,* 2021). Diabetes mellitus (DM) is a metabolic disease characterized by elevated blood sugar levels. Increase in blood sugar levels without control results in macro- and microvascular complications and a major cause of end-stage renal disease which leads to long term diabetes complications worldwide (Fagbohun *et al.,* 2020). The progression of diabetic complications is marked by renal structural abnormalities such as glomerular hypertrophy, mesangial matrix expansion, and thickening of a tubular glomerular basement with abnormal pathological values of albumin, creatinine in the plasma, and kidneys as well as that of the liver function tests (Vleming *et al.,* 1997; Alsaad and Herzenberg, 2007).

METHODOLOGY
Collection and Identification of the Plant:
The root of the plant was collected from villages around Dutsin-Ma Local Government, Katsina state, Nigeria. Botanical identification was done at Botany unit of Federal University Dutsin-Ma.

Preparation of the Methanolic Root Extract
Roots of the plant collected were air-dried under shade at the Biochemistry laboratory. The dried root of the plant was pounded using mortar and pestle, then sieved. Approximately 200 g of ground sample was dissolved in 1000 ml of methanol and left for 48 hours with periodic stirring. The solution was filtered using Whatman No 1 filter paper and the filtrate was placed in an ovum at 80°C for 8 hours to dry completely.

Experimental Animal
Twenty mixed rats weighing 60-100g were purchased from ABU Zaria Veterinary Department, Faculty of

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How to cite: Abdu, A.M, Said S.S, and Abdullahi, S.I.. (2022). Acute Toxicity Studies and Anti-diabetic Activity of Methanolic Root Extract of *Kigelia africana* (lam.) Benth in Alloxan Induced Diabetic Rats. UMYU Scientifica, 1(1), 90 – 97. https://doi.org/10.56919/usci.1222.011

Veterinary Medicine Kaduna State, Nigeria. The rats were acclimated for two weeks. Rats were given a starter mesh with unlimited access to water. They were kept in well-ventilated cages at the Animal Facility of the Federal University of Dutsin-ma, Katsina State.

Induction of Diabetes

Induction of diabetes with alloxan was performed intraperitoneally with 100 mg/kg body weight of alloxan and diabetes was confirmed 72 hours later.

Experimental design

Twenty (20) Wister albino male rats were assigned into 5 different groups which had four rats in each of the groups. Rats in each group were treated as follows:

- Group I: Non-diabetic, no treatment (Normal control).
- Group II: Diabetes induced rats without treatment (Negative control).
- Group III: Diabetes induced rats treated with standard drug (Glibenclamide 5 mg/kg).
- Group IV: Diabetes induced rats treated with methanol root extracts of *K. africana* (250 mg/kg b.w).
- Group V: Diabetes induced rats treated with methanol root extracts of *K. africana* (500 mg/kg b.w).

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

Acute Toxicity Study

The toxicity test was carried out using (8) Albino rats of both sexes. The animals were randomly grouped into five groups (I, II, III, and IV) of two animals per group. The animals were fed and had free access to water but were starved for 16 hours prior to testing. The extracts were orally administered as 50, 500, 1000, and 2000 mg/Kg methanolic extract of *K. Africana* methanolic root extract to Groups I, II, III and IV, respectively. The animals were observed continuously for the first 2 hours and then each hour for the next 24hours; and at 6 hours intervals for the next 48 hours after the administration of the plant extract to observe any death, external body surface changes or changes in general behavioral responses and also tremors, convulsion, salivation, diarrhea, lethargy, sleep, and coma of the animals according to the method described by Lorke, 1983.

Estimation of glucose levels

Serum glucose was estimated by glucose oxidase method (Barham, *et al.,* 1972) using Randox kit.

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) as described by Allain, *et al.,* (1974), High-Density Lipoprotein Cholesterol (HDL-C) as described by Burstein *et al.,* (1970) and Triglycerides (TAG) as described by Trinder., 1969. LDL-C and VLDL-C were calculated using Friedewald formula (Friedewald *et al.,* 1972),

- 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 as described by Reitman and Frankel., (1957).

Determination of serum electrolyte

Serum sodium and potassium were estimated by flame photometer as described by Harris., (1995). Serum chloride and bicarbonate were determined by the method as described by Kenkel., (2003).

Determination of serum urea

Serum urea was determined by enzymatic method as described by Fawcett and Scott., (1960); Chaney and Marbach., (1962).

Determination of liver function biomarkers

Plasma enzymes such as aspartate aminotransferase (AST), alkaline phosphatase (ALP) and alanine aminotransferase (ALT) were measured using Randox diagnostic kits. Total protein and total bilirubin (BIL) were also measured using the Randox diagnostic kit as described by Johnson *et al.,* (2014).

Phytochemical Screening of Methanolic Root Extract (Qualitative)

Test for Tannins

Braymer's test: 1mL of filtrate was mixed with 3mL of distilled water followed by 3 drops 10% Ferric chloride solution. The presence of blue-green colour indicates the presence of tannins (Singh and Kumar, 2017).

Test for Saponins

About 2 mg of Saponin in a test tube was dissolved in distilled water (2 ml). The mixture was shaken vigorously until frothing. A honeycomb-like frothing was formed after warming at 50°C for up to 15 min was indicative of saponin(Evans,2002).

Test for Flavanoids

A 1cm^3 of 10% NaOH was added to 3cm^3 of each extract in five test tubes. The presence of yellow coloration indicated the presence of flavonoids (Singh and Kumar, 2017).

Test for Alkaloids

1cm^3 of 1% HCL was added to 3cm^3 of each extract in five test tubes. The mixture was heated for 20 minutes. It was cooled and filtered. The filtrate was used for the test using Wagner"s reagent. Drops of Wagner"s reagent were added to 1cm3 of each extract. A reddish-brown precipitate indicates the presence of alkaloid in the extract (Singh V and Kumar R, 2017).

Test for Steroids

The test was carried out according to the method of Habon, 1973. About 1ml of the extract was dissolved in 2ml of chloroform, sulphuric acid [H_2SO_4conc] was carefully added to form lower layer. A reddish brown color at the interface indicated the presence of steroidal ring (1.e a glycone portion of the cardiac glycoside).

Cardiac Glycosides

1 ml of the extract was pipetted in 5 different test tubes followed by 1.5 ml glacial acetic acid. Then, 2ml of 3.5% ferric chloride solution was added and allowed to stand for 1 minute. One milliliter of concentrated H_2SO_4 was then carefully poured down the wall of the tube so as to form a cover layer. A reddish brown ring at the interface with the upper layer becoming green to blue indicated the presence of cardiac glycosides containing 2-deoxy sugar (Singh and Kumar, 2017).

Anthraquinones

Five gram (5g) of the plant extract was shaken with 10ml benzene, filtered and 5ml of 10% ammonia solution was added to the filtrate. The mixtures were shaken and the presence of a pink, red, or violent color in ammonical (lower) phase indicates the presence of free anthraquinones (Njoku OV and Obi C, 2009).

Volatile Oils

Small quantity of the extracts was shaken with dilute HCl. The absence of a white precipitate which was to be performed indicated the absence of volatile oils.

Statistical analysis

Obtained experimental test results are expressed as mean ± standard deviation (SD). Data were subjected to one-way analysis of variance (ANOVA) and differences between groups were determined by Tukey's multiple comparison tests using SPSS 16.0 (Social Science Statistics Program). The level of significance was set at $p< 0.05$.

RESULTS

Serum glucose level

Table 1 shows the effect of *K. africana* methanol root extract on fasting blood glucose (FBG) levels measured on days 3, 6, 9, 12, and 15 after induction and compared to normal and diabetic controls. The result showed a significant increase ($P<0.05$) in FBG levels in diabetic induced rats compared to normal rats. Oral administration of the extract at doses of 250 mg/kg and 500 mg/kg b.w resulted in FBG levels in the treated groups that is not significantly ($p<0.005$) different from the normal control rats on the 9th, 12th and 15th day. Groups given 5 mg/kg body weight orally of glibenclamide showed normal blood glucose levels.

Table 1: Effect of *K. africana* methanolic root extract on glucose level of alloxan induced diabetic rats.

Group Treatment	Before Induction	48 hours After induction	3rd day	6th day	9th day	12th day	15thday
GI NC	7.10±0.43^{a}	7.27±0.43^{a}	7.27±0.43^{a}	7.50±0.43^{a}	6.12±0.47^{a}	6.90±0.30	7.00±0.35^{a}
GII DC	6.60±0.45^{a}	10.10±0.46^{b}	11.95±0.18^{c}	12.72±0.46^{c}	14.75±0.33^{b}	15.20±0.48^{b}	15.40±0.21^{b}
GIII GLB 5mg/kg B.W.	7.53±0.70^{a}	11.17±1.00^{b}	10.33±1.65^{b}	9.26±1.73^{ab}	7.20±0.98^{a}	7.56±0.97^{a}	6.80±0.30^{a}
GIV KARE 250mg/kg B.W	6.00±0.56^{a}	10.30±0.46^{b}	10.15±0.33^{b}	9.77±0.49^{ab}	7.80±0.31^{a}	7.20±0.42^{a}	6.80±0.42^{a}
GV KARE 500mg/kg B.W	6.80±0.79^{a}	11.05±0.59^{b}	11.05±0.59^{ab}	9.67±0.94^{b}	7.70±0.36^{a}	6.70±0.54^{a}	5.90±0.38^{a}

Values with different alphabetical superscript along a column are significantly different at $P<0.05$, n = 4. KARE = *K. africana* root extract, GLB= Glibenclamide. NC= Normal control, DC= Diabetic control

Lipid profile

Table 2 shows that serum levels of total cholesterol, triglycerides, and LDL cholesterol were significantly increased ($P < 0.05$), while HDL was significantly decreased ($P < 0.05$) in diabetic controls compared to normal controls. The standard drug administered group (GIII) shows a significant reduction ($P < 0.05$) in T.CHOL, TG, and LDL compared to the diabetic control group. The extracts treated group shows a decrease in serum level of Total cholesterol TG and LDL cholesterol when compared with diabetic control group. The extract administered group (GV) showed significant increase in serum level of HDL-cholesterol.

Table 2: The effect of *Kigelia africana* root extract on the lipid profile of alloxan induced diabetic rats.

Groups/Treatment	T.CHOL mMol/L	HDL mMol/L	TG mMol/L	LDL mMol/L
G I NC	3.50±0.15^{a}	0.77±0.06^{b}	1.17±0.02^{a}	2.20±0.09^{a}
GII DC	4.10±0.05^{b}	0.36±0.16^{a}	1.43±0.14^{b}	2.90±0.05^{b}
GIII GLB (5mg/B.W)	3.56±0.12^{a}	0.93±0.03^{b}	0.96±0.06^{a}	2.16±0.12^{a}
G IV KARE (250mg/kg B.W)	3.35±0.13^{a}	0.77±0.10^{b}	1.02±0.07^{a}	2.08±0.06^{a}
G V KARE (500mg/kg B.W)	3.40±0.08^{a}	0.90±0.07^{b}	1.05±0.06^{a}	2.04±0.05^{a}

Values with different alphabetical superscript along a column are significantly different at P<0.05, n = 4. KARE = *k. africana* root extract, GLB= Glibenclamide. NC= Normal control, DC= Diabetic control

Haematological parameters

Results on table 3 shows significant increase packed cell volume (PCV) of diabetic untreated group when compared to the normal control group, while there was a significant decrease in white blood cell (WBC) and lymphocytes (LYMPH) levels of diabetic untreated group when compared to the normal group.

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Table 3: Effect of root extract of *K. africana* on Haematological Parameters of alloxan induced diabetic rats

Group/Treatment	PCV	HB	WBC	NEUT	LYMPH
G I NC	44.00±0.94^{ab}	12.92±0.40^{a}	4.45±1.25^{ab}	25.50±5.38^{a}	72.00±7.49^{b}
G II DC	35.25±1.08^{b}	11.85±1.10^{a}	2.50±0.20^{a}	23.66±1.85^{a}	64.66±2.60^{ab}
G III GLB	38.33±0.33^{a}	12.80±0.10^{a}	6.80±0.26^{bc}	31.33±6.38^{a}	68.66±6.38^{ab}
G IV KARE (250mg/kg B.W)	39.25±0.42^{a}	13.20±0.42^{a}	8.62±1.13^{c}	46.75±4.30^{b}	53.00±4.50^{a}
G V KARE (500mg/kg B.W)	41.00±1.73^{ab}	13.00±0.58^{a}	8.60±0.60^{c}	32.50±2.46^{a}	67.25±2.56^{ab}

Values with different alphabetical superscript along a column are significantly different at P<0.05, n = 4. KARE = *K. africana* root extract, GLB= Glibenclamide. NC= Normal control, DC= Diabetic control, HB= Haemoglobin, WBC= white blood cell, NEUT= Neutrophiles, LYMP= Lymphocytes.

Result of Kidney function test

The kidney function test results revealed that the serum level of all the kidney parameters have shown no significant changes (P<0.05) throughout the intake of the root extract when normal control group was compared with diabetic untreated group. There was significant difference in the serum concentration of Creatinine and urea in groups fed with 250mg/kg KARE and 500mg/kg.

Table 4: Kidney function test after 14 days of administration of root extract of *K. africana*

Group Treatment	Na^{+}	K^{+}	Cl^{-}	$HCO3^{-}$	Urea	Creatinine
NC	134.25 ± 2.06^{a}	3.32 ± 0.11^{ab}	91.25 ± 2.49^{a}	26.25 ± 0.85^{a}	8.14 ± 0.14^{ab}	77.00 ± 3.39^{ab}
DC	135.75 ±1.93^{a}	3.35 ±0.13^{ab}	94.00 ±1.63^{a}	24.00± 0.41^{a}	9.10 ±0.40^{ab}	76.25 ±1.93^{ab}
5mg/Kg GLB	103.75 ±34.59^{a}	2.45 ±0.83 ^{a}	72.75 ±24.26^{a}	18.50± 6.18^{a}	6.51± 2.19^{a}	56.00 ±18.80^{a}
250mg/Kg KARE	139.75± 0.75^{a}	3.72 ±0.11^{b}	100.75 ±2.69^{a}	24.25 ±0.63^{a}	9.67 ±0.25^{ab}	84.00 ±4.08^{ab}
500mg/kg KARE	138.50 ±1.71^{a}	4.35 ±0.21^{b}	103.00 ±1.73^{a}	76.75 ±51.75^{a}	10.27 ±0.19^{b}	86.50 ±2.63^{b}

Values with different alphabetical superscript along a column are significantly different at P<0.05, n = 4. KARE = *K. africana* root extract, GLB= Glibenclamide. NC= Normal control, DC= Diabetic control.

Result of liver function test

Table 5 shows the biochemical change in liver as diabetic rats have no any increase in enzyme parameters compared to normal (p<0.05). After treatment with the extract, the serum TP, SGOT, SGPT, and CB rats with dosage of 250mg/kg and 500mg/kg of the extract shows no significant difference (p<0.05) than the diabetic

group. The serum concentration of ALB and ALP showed no significant difference in comparison with the normal and diabetic control in the rats

Table 5: Liver function test after 14 days of administration of root extract of *Kigelia africana.*

Group Treatment	TB	CB	SGOT	SGPT	ALP	ALB	TP
NC	2.03±0.1[a]	0.90± 0.07[a]	10.00±0.[b]	10.50 ±0.96[a]	93.50±3.86[b]	3.60±0.16[ab]	18.98±13[a]
DC	1.75±0.1[a]	0.70± 0.13[a]	11.75±0.[b]	9.50± 0.50[a]	88.25±2.84[b]	4.18±0.10[ab]	6.45 ±0.21[a]
5mg/Kg GLB	1.35±0.4[a]	0.63± 0.21[a]	7.50± 2.5[a]	6.00± 2.00[a]	65.25±22.0[a]	2.98 ±0.99[a]	4.60± 1.54[a]
250mg/Kg KARE	2.15±0.1[a]	0.88± 0.13[a]	11.00±0.[b]	11.00± 0.58[a]	104.25±6.8[b]	4.56 ±0.25[b]	6.10 ±1.3[a]
500mg/kg KARE	2.13±0.3[a]	0.85 ±0.13[a]	8.5±0.50[b]	27.5 ±17.50[a]	96.25±5.17[b]	4.23±0.20[ab]	5.80 ±0.27[a]

Values with different alphabetical superscript along a column are significantly different at P<0.05, n = 4. KARE = *K. africana* root extract, GLB= Glibenclamide. NC= Normal control, DC= Diabetic control.

Phytochemical Analysis.

Table 6: Phytochemical (qualitative) analysis of methanol root extract of *K. africana*

Phytochemicals	Methanol Extract
Flavonoids	+ + +
Tannins	+ +
Alkaloids	+ + +
Anthraquinones	+
Cardiac Glycosides	+ +
Saponins	+ + +
Steroids	+ +
Volatile Oils	–

KEYS:- = Not present, + = Present in low amount , ++ = Present in moderate amount and +++ = Present in high amount

Table 7: Acute toxicity test of the oral administration of *k. africana* methanolic root extract on albino rat.

Group	Limit Test	Observation Period	Sign of Toxicity	Mortality
Group I	50mg/kg	28 days	Nill	0
Group II	500mg/kg	28days	Nill	0
Group III	1000mg/kg	28days	First rat becomes delibated, having difficulty in breathing and also bringing out fluids through the nose. The rat died at the same day.	2
Group IV	2000mg/kg	28days	Body system failure, and shrinkage leading to death	2

DISCUSSION

A phytochemical screening of the root extract of (*Kigelia africana*) found it to contain several secondary metabolites. The results of the current study, phytochemical screening, show the presence of flavonoids, alkaloids, tannins, saponins, cardiac glycosides, anthraquinones, and steroids in the methanol extract, as also obtained by Said & Co, (2019). Reports have indicated the pharmacological properties of these phytochemicals (Hassan *et al.,* 2011).

An acute toxicity study reveals no toxicity of the extract at dose of 50 and 500mg/kg. However, toxicity was observed due to the high dosage of administration at 1000 and 2000mg/kg which leads to mark elevation of body system changes and failure of organ functions, convulsions or coma were observed and mortality was recorded. Hence, the LD_{50} was observed at 1000 and 2000mg/kg.

The result of this research showed that after 15days of treatment with methanolic root extract of *K. africana* on diabetic rats there was significant reduction effect ($p<0.05$) on their blood glucose level (Table 1). The treatment group (GIV-GV) shows significant ($p<0.05$) reduction in blood glucose level at all doses of *K. africana* root extract, group III also shows similar reduction effect ($p<0.05$) against the standard drug. This is an indicative of a positive effect of *K. africana* root extract in reducing the blood sugar level, this result is in conformity with the research of Said *et al.,* (2019). Diabetic rats have significantly elevated sugar levels (hyperglycemia). This result is consistent with the existing literature that alloxan induces diabetes by selectively destroying pancreatic beta cells, leading to the marked increase in blood glucose concentration observed in rats after administration which confirms the manifestation of diabetes. (Akindele *et al*, 2012).

The significant difference ($p<0.05$) in serum lipids level observed between diabetic induced rats and normal rats might be associated with the disturbance in the regulation of the activity of the hormone-sensitive enzyme, lipase, due to insulin deficiency or absence, as a result of destruction of beta islet cells by alloxan (Hauwau, 2014). The plant extract has showed a reduction in T cholesterol which might be due to inhibition of fatty acid synthesis (Kumar *et al*., 2011). The plant extract might also have the potential to increase the reverse cholesterol transport pathway and reduced cholesterol concentration from the intestine as a result of α- glucosidase inhibition (Uhuo *et al*., 2019) The T.CHOL, TG and LDL-lowering effects coupled with the HDL-raising effects of KARE extracts may help reduce complications associated with hyperlipidemia secondary to diabetes mellitus (Chase, 2002 and Said *et al.,* 2019).

There was a significant decrease ($P<0.05$) observed for PCV level, WBC and LYMP when comparing diabetic untreated group with normal control group. Decrease in PCV could be due to a reduced red blood cell count (anemia)
as a result of decreased level of hemoglobin concentration. Persistent hyperglycemia might caused cardiac damage through changes in hematological parameters (Thomas *et al.,* 2003). Likewise, the decrease observed in WBC of diabetic control rats might be as a result of an increase in inflammation (Basiru *et al.,* 2022). Also, 250mg/kg B.W and 500mg/kg B.W of KARE has showed significant elevation ($P<0.05$) in PCV levels as well as WBC and LYMP when compared to diabetic untreated group (Table 3). 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 Ojo *et al*., (2020). Hence, the diabetic rats treated with *K. africana* methanolic root extract reversed some possible abnormalities and may prevent anemia. This is in agreement with an earlier statement by Ojo *et al*., (2020).

Electrolytes, creatinine and urea are markers of kidney function (Oduola *et al*., 2007). At the end of the research, no significant changes ($P<0.05$) was observed in all the kidney parameters throughout the intake of the root extract when normal control group was compared with diabetic untreated group. This might be because the alloxan did not stay longer to cause kidney damage. Estimation of serum creatinine along with blood urea is mostly used as diagnostic test to assess kidney function. However, the excretion of serum creatinine is rather constant and is not influenced by body metabolism or dietary (endogenous and exogenous) factors, as is the case with urea which is influenced by the protein content of the diet.

After treatment with the extract, the non significant difference ($p<0.05$) observed in serum ALB ,ALP, TP, SGOT, SGPT, and CB of treated group when compared to diabetic group might suggest that the extract might not affect the biliary function. Likewise, no significant difference ($p<0.05$) that was observed for serum TB which might indicates that diabetics did not prolong to have deleterious effect on the liver (Obianagha *et al.,* 2021).

CONCLUSION

At the end of this study, *K. africana* methanolic root extract was found to posses' blood glucose lowering activity, and have no toxic effect at very low dosage.

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