



## Screening of Anti-hyperglycemic Activity of *Kigelia africana* on Alloxan-Induced Diabetic Rats

### KEYWORDS

Alloxan, Anti-hyperglycemic, Glibenclamide, *Kigelia africana*

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**ABSTRACT** The present study was carried out to evaluate the anti-hyperglycemic activity of methanol extract of *Kigelia africana* leaves in Alloxan-induced diabetic rats for its purported use in diabetes. Hyperglycemia was induced in rat by injection of Alloxan (120 mg/kg, i.p.). Treatment was done by methanolic leaves extract of *Kigelia africana* (KA-ME) at dose range of 100–400 mg/kg, p.o for 21 days. Control group received normal saline (0.9%) for 21 days. Glibenclamide (5 mg/kg, p.o) was used as a reference drug. Blood samples were collected from all the groups and analyzed for serum glucose and lipid levels such as total cholesterol (TC), triglyceride (TG), proteins (TP). KA-ME was also tested for oral glucose tolerance test (OGTT) in normal fasted rats. KA-ME (200 & 400 mg/kg, p.o) showed a significant ( $P < 0.01$ ) reduction of serum glucose TC, TG, and TP level in Alloxan-induced diabetic rats as compared with diabetic control. KA-ME (200 & 400 mg/kg, p.o) significantly ( $P < 0.01$ ) increased the glucose tolerance in OGTT. The results obtained from the present study revealed the potential anti-hyperglycemic activity of methanolic extract from the leaves of *Kigelia africana*.

### INTRODUCTION

Diabetes mellitus is a leading metabolic disorder characterized by fasting and/or postprandial state hyperglycemia, resulting from defects in insulin secretion or action. It is well reported that diabetes mellitus is associated with a large number of macro vascular and micro vascular complications such as obesity, hypertension, hyperlipidaemia, nephropathy and neuropathy.<sup>[1], [2]</sup> A growing body of research has suggested that diabetes mellitus is increasing in an epidemic proportion throughout the globe, especially in India. Moreover, the prevalence of diabetes is expected to increase by more than two-fold worldwide and approximately 57 million Indians would be affected by this disorder in the year 2025, illustrating the severity and impact of the disorder on the quality of life.<sup>[3], [4]</sup> Despite the steady increase in the number of anti-diabetic agents, the prevalence of the disorder remains stable, may be due to the inconsistent efficacy of currently available drugs. In addition, the currently available anti-diabetic drugs have a large number of adverse effects and high rates of secondary failure.<sup>[5]</sup> Therefore, this remains a grave need to develop and discover new therapy with a proper balance of risk to benefit that could be fruitful for the treatment of diabetes mellitus. In recent decades, many researchers have sought new plant products to treat diabetes mellitus, as they contain many bioactive substances with therapeutic potential.<sup>[4]</sup>

*Kigelia* is a genus with only one species, *Kigelia africana*, belonging to family Bignoniaceae, popularly called the sausage tree, is a spreading tree bearing long, pendulous racemes of mottled dark flowers which appear like a candelabra<sup>[6]</sup>. This species can reach 66 feet (20 meters) in height. Its fruits are long and woody, sausage like in appearance with long cord-like stalks. Not uncommon, it is found in several areas of the city and few can miss it because of its uniquely shaped fruit - long cylindrical with a woody epicarp. It is evergreen in areas with abundant rainfall year round, but deciduous, losing its leaves seasonally. *Kigelia africana* is also grown as an ornamental plant, especially in tropical areas of the world, for its interesting flowers and fruits. They have showy flowers, the 5-lobed calyx and corolla, and the didynamous stamens. The corollas are leathery, which resists shredding by the clawed wings of bats that visit and pollinate this species

in its native habitat in tropical West Africa. The flowers and the large, sausage-like indehiscent fruits that develop from them hang on long stems below and away from the limbs of the tree. Although not edible, the fruits are used in Africa as an external medication.<sup>[7]</sup>

*Kigelia* is a native of W. Africa. The *K. africana* plant has many medicinal properties due to the presence of numerous secondary metabolites. These compounds include irridoids, flavonoids, Naphthoquinones and volatile constituent's etc. Because of these secondary metabolites present in plants, they may provide a basis for its traditional uses, particularly if they are the same as, or similar in structure to compounds from other species which display relevant activity. To some extent, the type of compounds likely to be present can be deduced from its taxonomic position and this can be seen to be the case with *Kigelia africana* which is a member of the Bignoniaceae.<sup>[8], [9]</sup>

### MATERIALS AND METHOD

#### Animals

Wistar rats, of either sex, weighing 150–250 g were used. They were housed under standard conditions of temperature ( $23 \pm 2^\circ\text{C}$ ), humidity and dark–light cycle (lights on from 6:00 am to 6:00 pm). Tap water was available at libitum. All the animals were carefully monitored and maintained in accordance with CPCSEA guidelines on control and supervision of experimental animals. The ethical clearance was obtained from the Institutional Animal Ethics Committee (Approval no.651/02/c/CPCSEA) before the experiment.

#### Chemicals

All chemicals and solvents used were of analytical grade, from S.D Fine Chemicals Ltd, Mumbai, India. Alloxan was purchased from Sigma Chemical Co. (St Louis, MO, USA) and Glibenclamide (Aventis Pharma Limited, Verna, Goa). Diagnostic kits of total cholesterol, triglycerides, and total proteins (Agappe Diagnostics Ltd., Pattimattom, Ernakulum), and rest all other reagents and chemicals were of analytical grade.

#### Plant Material and Preparation of Plant Extract

Sample of the leaves of *Kigelia africana* were collected from

the University of Pune. The samples were authenticated from Mr. Chakraborty, Botanical survey of India, Pune, and Maharashtra. The collected leaves of *Kigelia africana* was dried under shade for 10 days and then made into a coarse powder. Initially, 400 g of dried leaves was defatted with petroleum ether (60–80°C) in soxhlet apparatus (continuous hot percolation process) and after complete extraction (46 h), the solvent was removed by distillation under reduced pressure and resulting liquid was dried using heating plate at 50°C to get semisolid residue. After the extraction with petroleum ether, the same plant material was dried and further extracted with chloroform (36 h) followed by methanol (75 h) until the extraction was complete. The methanolic leaves extract was concentrated under reduced pressure and dried using heating plate at 60°C to get semisolid residue or respective residue.

#### Acute oral toxicity studies

The acute oral toxicity studies<sup>[10]</sup> of extracts were carried out as per the OECD guidelines, draft guidelines 423 adopted on 17 December 2001 received from CPCSEA, Ministry of Social Justice and Empowerment, Government of India. Administration of the stepwise doses of the methanolic extract of *Kigelia africana* from 50 mg/kg b. wt. up to a dose of 4000 mg/kg b. wt. caused no considerable signs of toxicity in the tested animals. One-tenth of the upper limit dose was selected as the level for examination of Anti-hyperglycemic activity.

#### Oral Glucose Tolerance Test

OGTT was performed in non-diabetic rats. The fasted rats were divided into 4 groups (n = 6/group). Group I: glucose load control group. Group II, III and IV rats received KA-ME at a dose of 100, 200 & 400 mg/kg body weight, respectively. The rats of treatment groups were loaded with glucose (2 g/kg, p.o.) 30 min after the administration of the KA-ME. Blood samples (100–200 µL) were collected at 0 min before the glucose load and 30, 60 and 120 min after the glucose load by retro-orbital vane plexus puncture under mild ether anaesthesia. The serum was separated and the glucose concentration was estimated by glucose oxidase-peroxidase (GOD-POD) method.<sup>[11]</sup>

#### Study of the *Kigelia africana* leaves extract on the Alloxan-induced hyperglycemia

The cytoprotective effects of extract were studied in Alloxan-induced diabetic rats according to the method reported.<sup>[12]</sup> The diabetes was induced by administration of 120 mg/kg Alloxan monohydrate (Sigma). The diabetic rats (glucose level > 275 mg/100 ml) were divided into six groups of six rats each. Group I served as negative control and received distilled water. Groups II served as the diabetic control, Group III, IV, V received the KA-ME at doses of 100, 200 and 400 mg/kg as an aqueous solution, p.o. and Group-VI received the Glibenclamide 5mg/kg as standard drug. The administration of the extract was continued for 21 days, once daily. Blood samples were collected from the retro-orbital plexus on days 1, 15 and 21 of extract administration. The blood glucose levels, triglyceride level, total cholesterol and total proteins were determined for all the samples by the glucose oxidase method.

#### Blood Collection

The blood samples (500–750 µL) were collected by retro-orbital vein plexus puncture of anaesthetized mice. Blood samples were collected at the time of grouping of animals (basal reading) and at 1st, 15th, and 21st day of treatment. Blood was centrifuged at 3500 r.p.m. for 20 min and serum was separated for biochemical estimation.

#### Estimation of Serum Glucose

The glucose concentration was estimated by (GOD-POD) method<sup>[13]</sup> using commercially available kit. The absorbance and concentration of test and standard samples were noted against blank at 505 nm with an autoanalyser.

#### Estimation of cholesterol

Serum total cholesterol was estimated by cholesterol oxidase-peroxidase (CHOD-POD) method<sup>[14]</sup> using commercially available kit.

#### Estimation of triglycerides

Serum triglyceride was estimated by glycerophosphate oxidase-peroxidase (GPO-PAP) method by the addition of enzyme present in reagent kit.<sup>[15]</sup> The absorbance and concentration of test and standard samples were noted against blank at 505 nm with an autoanalyser.

#### Estimation of total proteins

Serum proteins was estimated by Biuret method using commercially available kit.<sup>[16]</sup> The absorbance and concentration of test and standard samples were noted against blank at 546 nm with an autoanalyser.

#### Statistical analysis

Data were expressed as the mean ± S.E.M. The significance of the results was calculated using ANOVA and post hoc Dennett's t-test and the results were considered statistically significant when P<0.05.

## RESULT

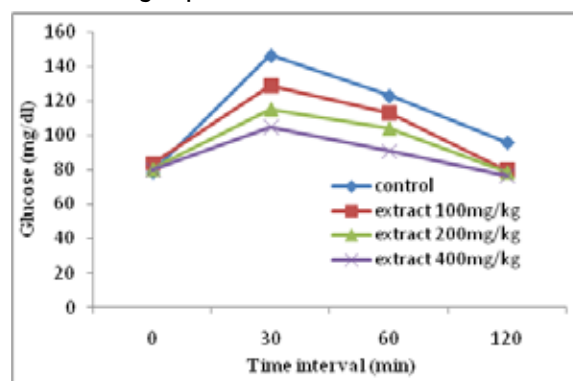
#### Oral Glucose Tolerance Test

The effects of KA-ME (100–400 mg/kg, p.o) on OGTT are summarized in Table 1 & Figure 1.

**Table 1: Effect of extract on oral glucose tolerance test in rats**

Groups	0 min	30 min	60 min	120 min
Vehicle control	78.33 ± 2.3	146.43 ± 1.7	122.87 ± 3.6	95.76 ± 1.9
Extract dose	83.59 ± 2.64	128.87 ± 1.1 ***	112.79 ± 3.4 ***	79.54 ± 1.7 ***
Extract dose II	80.21 ± 3.5	114.96 ± 2.82 ***	103.87 ± 2.3 ***	78.39 ± 1.5 ***
Extract dose III	79.95 ± 2.4	104.65 ± 3.7 ***	90.65 ± 4.29 ***	76.58 ± 1.45 ***

Values are presented as mean ± SEM. (n=5), ANOVA followed by Dunnett test. \*p<0.05, \*\*p<0.01 when compared with Control group.



**Figure 1: Effect of extract on blood glucose concentrations in fasting conditions at 0 min (pre-treatment) and 30, 60 and 120 min after oral glucose load in normal control rats, and rats treated with various doses of the methanolic leaves extract.**

Maximum serum glucose level was found at 30 min. in all groups after glucose load. The control group had a signifi-

cant elevation in serum glucose level throughout the total measurement period, i.e., for 120 min, with respect to KA-ME treatment group as shown in Figure 1. However, in the KA-ME extract treated groups, blood glucose level although it reached the peak level within 30 min of administration of glucose but it almost resettled to the normal level by 120 min. The glucose level significantly ( $P<0.01$ ) resettled close to the normal value in KA-ME (200 mg/kg and 400 mg/kg) treated group. Moreover, at the doses of 200 mg/kg & 400 mg/kg, the glucose level was significantly ( $P<0.01$ ) less as compared with glucose loaded control rats throughout at 120 min. However, no significant affect was observed at a dose of 100 mg/kg.

### Alloxan-induced Diabetic Rats Effect of KA-ME on serum glucose level

Table 2 summarizes the serum glucose levels in normal and diabetic rats. KA-ME at a dose range of 100–400 mg/kg decreased serum glucose levels in diabetic rats. The significant ( $P<0.01$ ) effect on serum glucose level was found at a dose of 400 mg/kg body weight, observed on 15<sup>th</sup> and 21<sup>st</sup> day of treatment. Moreover, the most pronounced decrease in serum glucose was observed on Day 21<sup>st</sup> at a dose of 400 mg/kg. In addition, the positive control Glibenclamide also significantly decreased the serum glucose level in diabetic rats as compared with diabetic control rats. However, KA-ME at a dose of 100 mg/kg failed to reach the level of significance as compared with diabetic control rats.

**Table 2: Effect of methanolic extract of *Kigelia africana* on serum glucose levels in alloxan-induced diabetic rats**

Groups	Serum glucose (mg/dl)		
	1 <sup>st</sup> day	15 <sup>th</sup> day	21 <sup>st</sup> day
Normal	89.41±5.42	91.15±3.89	85.12±5.71
Diabetic	308.8±5.27 <sup>##</sup>	299.5±6.55 <sup>##</sup>	302.64±8.54 <sup>##</sup>
Glibenclamide (mg/kg)	299.74±8.9	159.67±7.87 <sup>**</sup>	152.3±9.67 <sup>**</sup>
Extract (100mg/kg)	297.5±8.16	240.56±6.81 <sup>**</sup>	231.51±11.14 <sup>**</sup>
Extract (200mg/kg)	299.5±7.3	202.65±6.92 <sup>**</sup>	197.67±7.75 <sup>**</sup>
Extract (400mg/kg)	298.7±8.51	179.87±6.8 <sup>**</sup>	162.5±7.55 <sup>**</sup>

Values are presented as mean ± SEM. (n=5), ANOVA followed by Dunnett test. \* $p<0.05$ , \*\* $p<0.01$  when compared with Control group. <sup>##</sup> $p<0.01$  when Diabetic control compared with Normal group.

### Effect of KA-ME on serum triglyceride, cholesterol and protein levels

Table 3 illustrates the serum triglyceride, cholesterol and protein levels in normal and diabetic rats. Treatment of diabetic rats with KA-ME (200–400 mg/kg) produced a significant reduction in serum levels of triglyceride, cholesterol. The effect observed in our study was dose dependent and time dependent. Moreover, Glibenclamide treatment also significantly decreased the serum levels of triglyceride, cholesterol.

**Table 3: Effect of methanolic extract of *Kigelia africana* on Triglyceride, cholesterol and protein levels in alloxan-induced diabetic rats**

Groups	Days	Triglycerides (mg/dl)	Total cholesterol (mg/dl)	Total proteins (g/dl)
Normal group	0	83.17 ± 4.63	79.17 ± 4.62	6.33 ± 0.21
	15	89.69 ± 5.31	82.31 ± 5.19	6.38 ± 0.32
	21	87.71 ± 5.42	81.45 ± 3.72	6.22 ± 0.21
Diabetic control	0	84.75±6.69	75.63±4.56	6.45±0.32
	15	175.67±7.76 <sup>##</sup>	177.45±7.75 <sup>##</sup>	5.33±0.29 <sup>##</sup>
	21	187.51±8.46 <sup>##</sup>	183.66±5.77 <sup>##</sup>	5.12±0.29 <sup>##</sup>
Glibenclamide (mg/kg)	0	83.43±5.87	74.89±6.73	5.78±0.67
	15	138.56±6.75 <sup>**</sup>	143.64±6.39 <sup>**</sup>	5.72±0.64
	21	133.59±5.37 <sup>**</sup>	136.45±6.91 <sup>**</sup>	6.3±0.43 <sup>**</sup>
Extract (100mg/kg)	0	85.12±6.13	76.14±4.86	6.36±0.55
	15	165.66±7.09	161.38±5.89 <sup>**</sup>	5.4±0.48
	21	158.68±6.71 <sup>**</sup>	172.56±5.78 <sup>*</sup>	5.37±0.41
Extract (200mg/kg)	0	84.89±4.98	76.83±6.32	6.35±0.43

(200mg/kg)	15	157.71±6.54 <sup>**</sup>	156.7±6.32 <sup>**</sup>	5.48±0.39
	21	149.14±6.45 <sup>**</sup>	151.78±7.13 <sup>**</sup>	5.55±0.24
Extract (400mg/kg)	0	83.76±7.65	75.07±4.9	6.09±0.51
	15	144.77±6.19 <sup>**</sup>	146.65±6.38 <sup>**</sup>	5.63±0.58
	21	139.65±6.99 <sup>**</sup>	142.42±6.49 <sup>**</sup>	5.98±0.57 <sup>**</sup>

Values are presented as mean ± SEM. (n=5), ANOVA followed by Dunnett test. \* $p<0.05$ , \*\* $p<0.01$  when compared with Control group. <sup>##</sup> $p<0.01$  when Diabetic control compared with Normal group.

### DISCUSSION

Diabetes mellitus has been recognized as one of the most common metabolic disorders associated with common features such as hyperglycemia and hyperlipidaemia. Alloxan is a  $\beta$ -cytotoxin diabetogenic agent, which induces diabetes by destructing the  $\beta$ -cells of the islets of pancreas, leading to a decreased insulin release and increased blood glucose level.<sup>[17]</sup> In accordance with the previous findings, the present study reports the significant increase in serum

Glucose level in Alloxan-induced diabetic rats. The chronic administration (21 days) of the KA-ME produced a decrease in serum glucose levels of diabetic rats. This effect may be due to regeneration of the  $\beta$ -cell following destruction by Alloxan. The growing body of data suggested that to achieve maximum effect, therapy with plant products should be continued for a longer duration.<sup>[18]</sup> Considering this, KA-ME was administered daily for 21 days, the period which may be produced a significant reduction in all the diabetic markers, and this effect was potent as compared to acute dosing.

In the present study we also investigated glucose tolerance test in normal rats. The KA-ME significantly decreased the serum glucose levels in glucose loaded rats, and this infor-

mation could be endorsed to the potentiating of the insulin effect of blood by increasing the pancreatic secretion of insulin from existing  $\beta$ -cells or its release from bound insulin.<sup>[19]</sup> In this context, a number of other plants have been observed to have similar pattern of hypoglycemic effects. Results on the insulin release from pancreas directly indicate that the anti-hyperglycemic activity of *Kigelia africana* may be through the release of insulin from the pancreas.

## CONCLUSION

Herbal hypoglycemic agents can provide better option to avoid harmful side effects caused by prolong intake of synthetic ones. From present preclinical studies, *Kigelia africana* proved to be hypoglycemic in action. But one can speculate that in clinical trials, the drug may act as safe and effective hypoglycemic agent. The remarkable hypoglycemic potential of *Kigelia africana* was quite competent with standard drug. Although the test drug could not correct deranged levels of serum metabolites, it can be used in polyherbal formulations. Further studies are necessary to elucidate details of active phytochemical and their mechanism of hypoglycemic action. Isolation and study of active principles are under process.

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