



## The Effect of *Kigelia Africana* Fruit Extract on Blood Glucose in Diabetes Induced Mice

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### Abstract

Objective of this study was to determine the effect *Kigelia africana* fruit extract has on blood glucose levels of diabetes mice and its phytochemical profile. Mice were induced with diabetes using Alloxan monohydrate 90mg/kg. Blood glucose was checked before induction and 72 hours after induction to confirm diabetes. Treatment involved using oral administration of *Kigelia* fruit extract 1000mg/kg, *Kigelia* fruit extract 500mg/kg, Glibenclamide 0.25 mg/kg, *Kigelia* fruit extract 500mg/kg and Glibenclamide 0.25mg/kg and Normal Saline. The results showed a greater reduction in blood glucose of mice after treatment with *Kigelia* extract 1000mg/kg compared to *Kigelia* 500mg/kg [(5.3 +/- 0.5mmol/l) vs (6.3+/- 0.6mmol/l), (p= 0.005)]. Further, Glibenclamide 0.25mg/kg showed less reduction in blood glucose than *Kigelia* 1000mg/kg [(7.4+/-0.9mmol/l) vs (5.3 +/- 0.5), (p= 0.00)]. The mean blood glucose levels were lower in mice that received *Kigelia* extract than those that received both *Kigelia* extract and Glibenclamide [(5.3 +/- 0.5mmol/l) vs (7.8 +/- 0.6 mmol/l), (p=0.00)]. The fruit extract tested positive for Tannins, Saponins, Flavanoids, Alkaloids, Glycosides and Steroids. Findings of this study indicate that *Kigelia africana* fruit extract causes reduction in blood glucose of diabetes induced mice and gives better results when used alone than in concomitant use with Glibenclamide. The study also indicates that the fruit extract has alkaloids, saponins, steroids, glycosides, tannins and flavonoids.

**Key words:** *Kigelia africana* fruit extract, antidiabetic, Alloxan monohydrate

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### Introduction

Diabetes mellitus, a syndrome of chronic hyperglycemia, claims about 4 million lives every year [7]. In Zambia this syndrome causes about 7,600 deaths yearly [7]. It was estimated that the cost of treating diabetes in the year 2005 in Zambia, using conventional medicine ranged from US\$ 2302 - US\$3207 per person [4]. It is for this reason that alternative remedies have to be found. *Kigelia Africana* (*Lam*) *Benth* (**Mupolota in silozi**) is a plant attracting interest among scientists and grows in many parts of the world.

In Asia, a study done on *Kigelia pinnata* (*Jacq*) (*syn*) *Kigelia africana* *Lam* (*Benth*) flowers showed potential anti-hyperglycemic properties in type II diabetic induced mice [8]. Uhuo et al., [12] conducted a comparative study of the effect of the stem and bark of the Nigerian plant on some biochemical parameters in diabetic induced rats and showed a drop in the fasting blood sugar of the diabetes induced rats. In a review of several documents, by Olatunji *et al* [11], it is mentioned that studies conducted by Houghton in 1983 showed a positive result for cytotoxic properties of fruit extract especially towards melanoma. Further, he mentions laboratory studies that show the presence of naphthoquinones. Ankuret *al* [2] showed that *Kigelia africana* extract has got positive activity against *pseudomonas aeruginosa*.

Though research is being done on parts of the plant in other parts of the world, little is published about the plant growing in Zambia especially that different habitations can affect the chemical composition of a plant. This made it necessary to look at the fruit since anecdotal evidence shows that the Zambian population uses the fruit for the purpose of treating diabetes. The need to explore its anti-diabetic properties would help us conclude if it

actually has hypoglycemic properties or not. Recent isolation of iridoids, alkaloid derivatives in the fruit growing in India suggests anti-hyperglycemic properties [6]. However, information as to whether these phytochemicals are present in *Kigelia* growing in Zambia is not available.

National policy to research traditional medicine in order to integrate them into conventional medicine made a study such as this one necessary because it answered the call to public policy that has been put by both the government and WHO [13].

## Materials and Methods

**Plant material:** Identification of the fruit was conducted at the University Of Zambia School Of Natural Sciences, Department of Biological Sciences. The fruit was harvested from Kazungula district, Southern Province of Zambia, Village Musokotwane and Chief Musokotwane in the month of December.

**Chemicals and apparatus:** Chemicals were obtained commercially and were of analytical grade. These included; Glibenclamide (Cipla), Alloxan monohydrate (sigma), Solvent (distilled water or methanol and dichloromethane) for extraction, Lignocaine solution 1% for local anaesthesia (Ranbaxy), Accu-check glucometer and glucose sticks, Test tubes, Feeding needles, Insulin needles and syringes, Lancets, Silica gel plates for Thin layer chromatographs TLC, Beakers, Buchner's funnel, electronic balance, mouse scale.

**Extract preparation:** The fresh fruit was cut into pieces and put into a blender for a fine consistency. About 100g of fruit was put into a large beaker and 150 mls of distilled water was added to collect the first extract after bringing it to the boil for about 5 minutes. The mixture was cooled and filtered using a Buchner's funnel through suction filtration. A series of five (5) extractions were done from the same fruit with the second through to the fifth extraction using 100mls of water. The crude extract was dried in an oven at 40°C and weighed and stored in a refrigerator at 10°C before use in the mice. Only the first aqueous extract was used for treatment since it had the most concentration of phytochemicals.

**Animals:** Laboratory albino mice (*Mus musculus* species) weighing between 18g and 35g were used. These mice were bred at the University of Zambia, Physiological Sciences department. A total of 35 mice picked using convenient sampling were used for the study. They were randomly grouped into five (5). The mice were kept in a place that was well ventilated, had adequate food, water and sunlight throughout the study. The

mice were fed a usual diet which comprises 45% fat, 35% protein and 30% carbohydrates as a percent of total Kilo calories. This feed was given throughout the study except a day before the induction of diabetes since the mice had to be fasted.

**Inducing diabetes:** The mice were allowed to acclimatize to the new environment for 24 hours before the study could begin and randomly placed into 5 groups. Mice were weighed and a baseline glucose level established. Diabetes was induced in all mice using Alloxan monohydrate 90mg/kg intraperitoneal. Random blood sugar was collected 72 hours after induction and used as a baseline. Treatment was started when mice had blood glucose of above 9.0mmol/l.

**Experimental designs:** Treatment was given per oral once daily, at the same time according to the desired dosage for each product used. Random blood sugars to monitor treatment were collected every alternate day for 14 days. A drop Blood from the mice was collected from the tail vein. The following are the groups of mice according to treatment that was given; Group 1- Mice are treated with 1000mg/kg *K. africana* extract PO once daily; Group 2 – Mice treated with 500 mg/kg *K. africana* extract PO once daily; Group 3- Mice treated with Glibenclamide 0.25mg/kg PO once daily only; Group 4- Mice treated with 500mg/kg plant extract and Glibenclamide PO once daily and Group 5- Mice treated with normal saline PO once daily. Blood sugar was measured using Accu check glucometer and glucose sticks.

Phytochemical screening was done for

1. Tannins: About 3 drops of 10% FeCl<sub>3</sub> was added to 2mls of fruit extract. A dark - blue precipitate was observed showing the presence of tannins.
2. Test for Saponins: About 5mls of crude extract was shaken vigorously with 5mls of distilled water in a test tube and warmed. Stable foam shows presence of saponins.
3. Test for Flavanoids: About 2 fragments of metallic Magnesium and 3mls extract were combined. Then, to this 0.5 ml of concentrated hydrochloric acid (HCl) was added. A red color shows presence of flavonoids.
4. Alkaloids: About 3mls of 1% HCl is added to 3mls fruit extract and put in a steam bath. Mayer's reagent was added to this mixture. A formation of a precipitate shows the presence of alkaloids.
5. Steroid test: About 1ml extract was dissolved in 3mls of chloroform and then filtered. To the

filtrate, concentrated Sulphuric acid [H<sub>2</sub>SO<sub>4</sub> conc] was added to form a lower layer. A reddish brown ring was noted to show the presence of steroids.

- Glycoside test: To 1ml of fruit extract, about 3mls glacial acetic acid containing 1 drop of 1% FeCl<sub>3</sub> (iron chloride) was added. This was under laid with concentrated Sulphuric acid. The presence of a green layer was observed, suggesting the presence of glycosides.

**Data analysis:** Blood glucose results for each group of mice were expressed as a Mean +/- Standard deviation. Since two different groups were being compared at a time, an independent t-test was used for data analysis. The phytochemical analysis was descriptive and presented as present or absent.

## Results

### Effect of Kigelia fruit extract on blood glucose

During the experiment, blood sugars were observed to be low before induction with diabetes than after induction. Mice that received treatment had their blood sugars reduce compared to those that

did not receive any treatment, (group 5), refer to figure 1. After treatment of the mice, the results showed a greater reduction in blood glucose of mice treated with Kigelia extract 1000mg/kg compared to Kigelia 500mg/kg, [(5.3 +/- 0.5mmol/l) vs (6.3 +/- 0.6mmol/l) , (p= 0.02)], this is seen in figure 2. Further, mice on Glibenclamide 0.25mg/kg showed less reduction in blood glucose than those on Kigelia 1000mg/kg [(7.4 +/- 0.9mmol/l) vs (5.3 +/- 0.5mmol/l), (p= 0.00)], seen in figure 3. Mice that received both extract and glibenclamide concomitantly showed a poor reduction in blood sugar compared to those that received either extract alone or glibenclamide alone [(7.8 +/- 0.6 mmol/l) vs (5.3 +/- 0.5mmol/l) or (7.4 +/- 0.9mmol/l), (p=0.00)], also seen in figure 4. The tables show readings that indicate; fasting blood glucose that was taken before induction of diabetes with Alloxan monohydrate (Table 1); random blood glucose 72 hrs after Alloxan monohydrate but before treatment hence serving as baseline (Table 2); and random blood glucose readings after 14 days of treatment (Table 3).

Group ID	KAFE* 1000mg/kg	KAFE* 500mg/kg	Glibenclamide 0.25mg/kg	Glibenclamide and KAFE*	Normal Saline
Mean	4.4	4.2	4.1	3.8	3.7
S.D**	+/-0.8mmol/l	+/-1.9mmol/l	+/-1.2mmol/l	+/-0.9mmol/l	+/-1.0mmol/l

**Table 1:** Average fasting blood glucose (FBG) of mice in five different groups.

KAFE\* - Kigelia Africana Fruit Extract

\*\* S.D- standard deviation

Group ID	KAFE 1000mg/kg	KAFE 500mg/kg	Glib 0.25mg/kg	Glib and KAFE	Normal Saline
Mean RBG	9.9	9.8	11.1	10.2	12.4
S.D**	+/-1.9mmol/l	+/-1.1mmol/l	+/- 3.6mmol/l	+/-2.7mmol/l	+/3.6mmol/l

**Table 2:** Average Random blood glucose (RBG) 72 hours after Alloxan injection

KAFE\* - Kigelia Africana Fruit Extract

\*\* S.D- standard deviation

Group ID	KAFE* 1000mg/kg	KAFE* 500 mg/kg	Glibenclamide 0.25 mg/kg	Glib +KAFE* 500 mg/kg	Normal Saline
Mean RBG +/- S.D**	5.3 +/-0.5mmol/l	6.3 +/-0.6mmol/l	7.4 +/-0.9mmol/l	7.8 +/-0.6mmol/l	19.1 +/-8.4mmol/l

**Table 3:** Average random blood glucose (RBG) reading with standard deviations on day 15 after treatment

\*KAFE- Kigelia africana fruit extract, Glib- Glibenclamide

\*\* S.D – Standard deviation

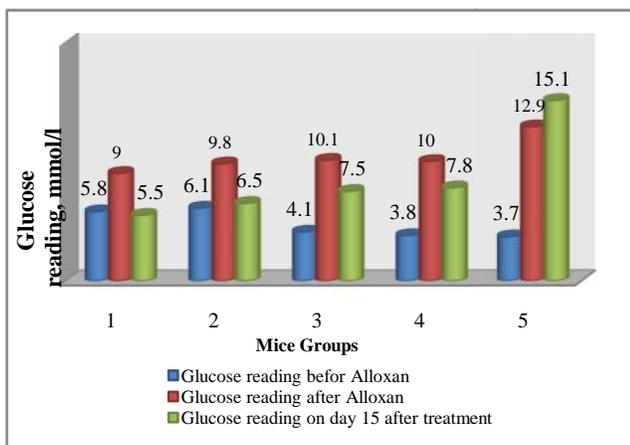
**Phytochemical analysis:** The fruit extract showed a positive result for the presence of alkaloids, saponins, tannins, flavonoids, steroids and glycosides. Since a series of extractions was conducted, the first 3 extractions showed

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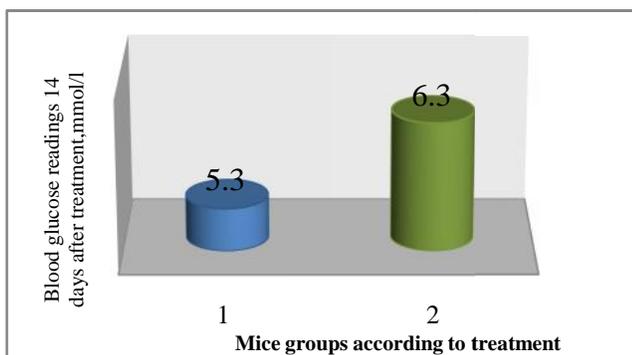
clearly tested positive for the particular phytochemical while the last two either had negative or somewhat positive result of the phytochemical. Hence with increased series of extraction, there were less active compounds present in the extract. The steroid and glycoside test was done mainly on the organic extract as the organic extracts dried the quickest. (Table 4)

Test / Extract Series	1	2	3	4	5
Tannins	++++	+++	++	+	+
Saponins	++++	+++	+	-	-
Flavanoids	++++	+++	++	+	+
Alkaloids	++++	+++	++	+	-
Steroid test for organic extract	Positive				
Glycoside test for organic extract					

**Table 4:** Results on phytochemical analysis showing decreasing concentration of phytochemicals with increase in extract series



**Figure 1:** Summary of blood glucose readings before and after treatment



**Figure 2:** Dose dependent reduction seen with KAFE 1000mg/kg vs 500mg/kg, [(5.3+/- 0.5 mmol/l vs (6.3+/- 0.6 mmol/l) p= 0.005]

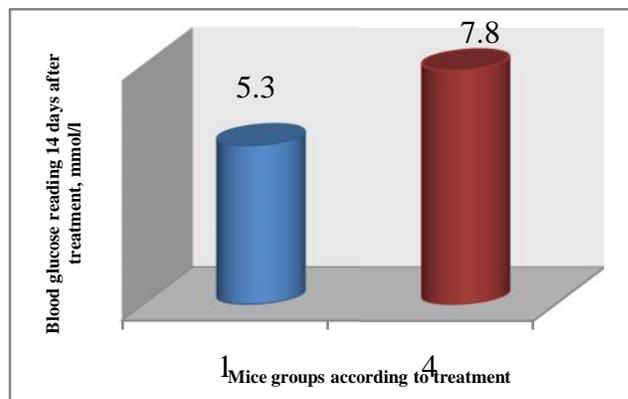
## Discussion

The phytochemical analysis conducted on the fruit extract shows a presence of alkaloids, saponins, tannins, glycosides, steroids and flavonoids. In the case were a series of extractions was conducted, presence of the phytochemicals reduced with the increase in series. Olatunji and Colleagues’ review as

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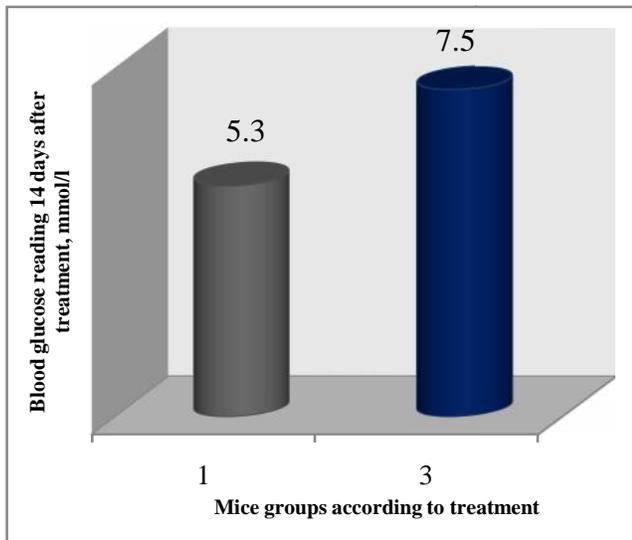
well as Amandeep and Colleagues [1] mention the presence of secondary metabolites such as phenols, flavonoids and alkaloids among others. Although these compounds are found in the different parts of the plants, Amandeep and Colleagues further mention that the fruit extract showed presence of glycosides, phenols and alkaloids and this agrees with the findings of this study, though the reagents used for the tests may have been different. Further, Nyarko and colleagues[10] mention that there is a strong association between the hypoglycemic properties of the plant and the presence of alkaloids and it was supposed that the plant extract has insulin secretogogical properties; this made a base for the use of Glibenclamide as a comparison to *Kigelia africana* fruit extract, since it also improves insulin secretion [5]. It is still important to isolate the individual compounds and relate them to those involved in the treatment of diabetes. The TLC conducted in this study provides a stepping stone for further chemical analysis that could be conducted to isolate active compounds.



**Figure 3:** Poor blood glucose reduction in concomitant use (*Kigelia* extract and Glibenclamide) than single use (*Kigelia* only),[(7.8+/- 0.6mmol/l vs 5.3 +/- 0.5mmol/l) p=0.000]

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**Figure 4:** Poor blood reduction with Glibenclamide than with KAFE 1000mg, [(5.3 +/- 0.5 mmol/l vs 7.5 +/- 0.9 mmol/l), p=0.00]

Another finding of this study was that daily administration of the extract for two weeks at two different doses leads to a dose dependent reduction in blood glucose levels of diabetic induced mice. By the 14<sup>th</sup> day of treatment there was a more statistically significant reduction in the average RBG level of mice on KAFE 1000 mg/kg (5.3 +/- 0.5 mmol/l) than in mice on KAFE 500mg/kg (6.3 +/- 0.6 mmol/l), (p=0.02). Although this study did not aim at getting the effective therapeutic dose, this result shows us that an increase in the dose gives a better blood glucose lowering effect. These results may be compared to those of Kumar and Colleagues [8] even though their study was conducted in rats that were induced with streptozotocin and also, flowers instead of the fruit were used. Further, Uhwo and Colleagues [12] also demonstrated a blood glucose lowering effect of the leaf and stem extract of *Kigelia* in diabetes induced rats. Similar to the study conducted by Kumar and Colleagues, Uhwo and Colleagues used rats though induced by Alloxan monohydrate like in this study. The reduction in the fasting blood glucose of rats conducted by Uhwo and colleagues was not a dose dependent reduction as only one dose of the extract was used. Much as this study shows a better reduction at doses as high as 1000mg/kg of the fruit extract, it is necessary to assess for possible maximum therapeutic dose so as to look out for toxic effects of the drug at very high doses, by looking out for changes in biochemical parameters at different doses.

In as far as what effect concomitant use of the fruit extract with glibenclamide would have on the blood glucose of the diabetes induced mice, the results obtained in this study show that after 14 days of treatment, there is a statistically significant reduction in the RBG of mice receiving *Kigelia africana* 1000mg/kg (5.3 +/- 0.5 mmol/l) than those receiving both *Kigelia africana* fruit extract 500mg/kg and glibenclamide 0.25mg/kg (7.8 +/- 0.6 mmol/l), (p=0.00), (fig.3). Glibenclamide has insulin secretogogical properties on residual beta cells of the pancreas, [5]. Though there is no established mechanism of action of the fruit extract, scholars that have studied this plant's effect on blood glucose of diabetic induced mice suggest that it also has insulin secretogogical properties [10]. It would be expected to observe a synergistic effect with the concomitant use of KAFE and Glibenclamide. Surprisingly, in this study it is seen that the concomitant use is inferior to the single use of either the fruit extract alone or the glibenclamide alone. It can therefore be argued that there is a possible pharmacologic interaction. However, this result shows that concomitant use of KAFE and glibenclamide is not likely to cause hypoglycemia in diabetes induced mice.

In summary, this study has shown that the fruit extract contains among other phytochemicals alkaloids and reduces blood glucose levels in diabetes induced mice. Further, the use of the fruit extract concomitantly with glibenclamide also reduces blood glucose levels in diabetic induced mice but does not cause hypoglycemia.

The use of chemical induced mice did not allow us to achieve an ideal type II diabetic model (db/db model). However, because of the partial destruction of the pancreas, we achieved a hyperglycemic model that had residual beta cells. An ideal model would mimic the physiologic state of a type II diabetic patient. Further, the mice had constant exposure to food during the study, this made it difficult to access such measures as the fasting blood sugars and post prandial blood sugar after exposure to meals.

The phytochemical analysis only gave a basic profile because of limitations with access to equipment needed to give isolation of compounds that could exist in the fruit extract.

## Conclusion

Findings of this study indicate that *Kigelia africana* fruit extract causes reduction in blood

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glucose of diabetes induced mice and gives a better result when used alone than in concomitant use with Glibenclamide. The fruit extract contains; saponins, alkaloids, flavonoids, tannins, steroids and glycosides.

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**Conflict of interests:** We declare that we have no conflicts of interest.

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