THE EFFECT OF KIGELIA AFRICANA FRUIT EXTRACT ON BLOOD GLUCOSE IN DIABETES INDUCED MICE

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A thesis submitted in partial fulfilment of the requirement for the award of the degree of Master of Clinical Pharmacy.

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DECLARATION

I hereby declare that this dissertation represents my own work and has not been presented either wholly or in part for a degree at the University of Zambia or at any other University.

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DEDICATION

This work is dedicated to my mum and dad, (Mr and Mrs Muyenga) who inspired it as well as my husband (Mr N. X. Akapelwa) for his unwavering support during the study.

ABSTRACT

Objective: To determine the effect *Kigelia africana* fruit extract has on blood glucose levels of diabetes mice and its phytochemical profile

Specific objectives:

1. To determine *Kigelia africana* fruit extracts' effect on blood glucose levels when used alone and concomitantly with Glibenclamide on mice.

2. To identify the basic phytochemical composition of the fruit extract.

Design: An analytical study involving aqueous and organic extraction of the fruit, conducting phytochemical analysis of the extract and treating diabetes induced mice bred in Physiological Sciences Department of University of Zambia with the extract.

Setting: Departments of Chemistry and Physiological Sciences of the University of Zambia

Method: 35 albino mice (18-30g) allocated in 5 groups of 7 according to treatment. **Group 1**- Kigelia fruit extract 1000mg/kg, **Group 2**- Kigelia fruit extract 500mg/kg, **Group 3**- Glibenclamide 0.25 mg/kg, **Group 4**- Kigelia fruit extract 500mg/kg and Glibenclamide 0.25mg/kg and **Group 5**- Normal Saline.

Aqueous and organic extracts were collected from fruit sample by boiling and maceration respectively and tested for tannins, saponins, flavonoids, alkaloids, glycosides and steroids.

Main outcome measures: RBS sugar of below 8mmol/l.

Results: 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.

Conclusion: 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.

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ACRONYMS AND ABREVIATIONS

CYP 450 CYTOCHROME P450

DM DIABETES MELLITUS

FBG FASTING BLOOD GLUCOSE

RBG RANDOM BLOOD GLUCOSE

KAFE KIGELIA AFRICANA FRUIT EXTRACT

GLIB GLIBENCLAMIDE

TLC THIN LAYER CHROMATOGRAPHY

RF value REFRACTIVE INDEX VALUE

SPSS STATISTICAL PACKAGE FOR SOCIAL SCIENCES

MSL MEDICAL STORES LIMITED OF ZAMBIA

WHO WORLD HEALTH ORGANIZATION

DEFINITIONS OF KEY WORDS

- **ADD-199 -** A herbal preparation that was made from *Maytenus Senegalensis*, *Annona Senegalensis*, *Kigelia Africana* and *Lannea Welwitschii*.
- **Alloxan monohydrate**- a chemical used to induce diabetes in mice.
- **Random blood glucose after treatment**- blood glucose level ranging from 2.9mmol/l to 9 mmol/l after treatment*
- **Conventional anti-hyperglycemic -** drugs used in the treatment of diabetes in modern medicine eg, metformin or glibenclamide
- **Diabetes induced mice**- mice that have Alloxan monohydrate injected in them to cause hyperglycemia and have a Random blood sugar of equal to or more than 9 mmol/l.
- **Hyperglycemia**- blood glucose level of ≥ 9 mmol/l*
- ***Kigelia africana* fruit extract** – fruit extract collected through extraction using 1:1 methanol and dichloromethane or distilled water.
- ***Mupolota***- traditional name for *Kigelia africana* among the Lozi speaking people in Zambia.
- **Solvent**- water, dichloromethane or methanol used for the process of extraction

***blood sugar levels were determined after comparing with other studies that induced diabetes in mice*

CHAPTER ONE

1.0. INTRODUCTION

1.1. Background

Diabetes mellitus, a syndrome of chronic hyperglycemia, claims about 4 million lives every year (IDF, 2013). In Zambia this syndrome causes about 7,600 deaths yearly (IDF, 2013). 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 (Beran *et al.,* 2005). 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 (Kumar *el al.,* 2012). In a review of several documents, by Olatunji *et al (2009),* 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 naphtoquinones. Ankur *et al* (2011) showed that *Kigelia africana* extract has got positive activity against *pseudomonas aeruginosa*. In India, a study was conducted to assess the phytochemical constituents of *Kigelia africana* fruit extract (Bhramaramba *et al*, 2012). Other studies conducted on parts of *Kigelia africana* plant growing in West Africa, showed that it has anti-inflammatory, antimicrobial as well as anti-malarial activities (Wilkinson, 2005.).

Due to its wide phytochemical composition, pharmacological research from several angles can be done, concerning several anecdotal medicinal uses that have been passed over the years. Evaluated literature showed that little has been done to assess the anti-diabetic properties of *Kigelia africana* in Zambia, as there is little available published literature concerning studies on the medicinal properties of *Kigelia africana* fruit **(Mupolota)** including its use in diabetes.

This study evaluated the effect of *Kigelia africana* fruit extract on blood glucose of diabetic induced mice. It aimed at providing evidence based information regarding the medicinal properties of the fruit extract.

1.2. Statement Of Problem

Currently, 347 million people worldwide have diabetes, Africa has 20 million diabetics while Zambia had about 266,000 cases of diabetes in the year 2014 (IDF, 2014). The cost burden is also not a light one, an insulin vial in 2011 ranged between 8.00 US dollars and 10.00 US dollars. According to the MSL (Medical Stores Limited, Zambia) bulletin of 2011, the average monthly consumption of soluble insulin and insulin lente was 486.6 vials and 431.1 vials respectively hence; the monthly average treatment cost was about 4,866 US dollars and 4311 US dollars respectively(MSL stock status as at 14th Oct 2011). If the cost of tackling such a growing problem using conventional medicine is so high on the government budget, it would be good to try alternative remedies that may be cheaper.

Kigelia africana is a plant that anecdotal evidence has shown to be highly recommended among some diabetic patients in Zambia to alleviate symptoms of diabetes. Early settlers who wrote books about medicinal plants in Zambia also mentioned *Kigelia africana* as one of the plants used in traditional medicine to treat diabetes by the locals (Fowler, 2007). If the above is true, there was need for more published information that discusses its use as a possible anti-diabetic. A picture of how the fruit extract would interact with conventional medicine in a patient taking both was also necessary.

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.

1.3. Rational of Study

Diabetes is a growing disease condition that will be the 7th leading cause of death in the world by 2030 (WHO, 2014). This also entails that treatment cost will also increase, thus a need to look for alternative medicines is apparent. Therefore, studies such as this one which explored the use of herbal medicines are of great significance.

Much as there is a wide use of *Kigelia africana* in the world, there is still much that needs to be known about it. The need to explore its anti-diabetic properties would help us conclude if it actually has hypoglycemic properties or not. Recent issolation of iridoids, alkaloid derivatives in the fruit growing in India suggests anti-hyperglycemic properties Hitesh *et al.,(2013)*. 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 (WHO, 2013).

1.4. RESEARCH QUESTION

What effect does *Kigelia africana* fruit extract have on blood glucose levels of diabetes induced mice and what phytochemical composition does *the* fruit extract have?

1.4.1. FOLLOW UP QUESTION: What effect would *Kigelia africana* fruit have on blood glucose levels when used concomitantly with Glibenclamide on mice models?

1.5 GENERAL OBJECTIVE

To determine the effect *Kigelia africana* fruit extract has on blood glucose levels of diabetes induced mice and the phytochemical composition of fruit extract.

1.5.1. SPECIFIC OBJECTIVES

1. To determine *Kigelia africana* fruit extracts' effect on blood glucose levels when used alone and concomitantly with Glibenclamide on mice.

2. To identify the basic phytochemical composition of *Kigelia africana* fruit extract.

Table 1 - <u>Conceptual variables</u>

Objectives	**Variable of measurement**	**Type of variable**	**Study indicator**	**Data source**
To determine *Kigelia africana* fruit extracts' effect on blood glucose levels in mice	Blood glucose	Continuous	Blood glucose below 8mmol/l	Blood of mice from tail vein
To assess *K. africana* fruit extracts' hypoglycemic effect when used concomitantly with Glibenclamide in mice.	Blood glucose	Continuous	Blood glucose below 8mmol/l	Blood of mice from tail vein
To identify the basic phytochemical composition of the fruit extract.	Phytochemicals	Dichotomous Categorical	Alkaloids Flavonoids Saponins Glycosides Steroids Tannins	Aqueous and organic fruit extract

CHAPTER TWO

2.0. LITERATURE REVIEW

This review of literature explored the major concerns centered on the effect of *Kigelia africana* fruit extract on blood glucose of diabetic induced mice. The review of literature will focus on objectives one and two, these being;

1. To determine *Kigelia africana* fruit extracts' effect on blood glucose levels when used alone and concomitantly with Glibenclamide on mice.
2. To identify the basic phytochemical profile of the fruit extract.

In a review written by Olatunji *et al,* (2009) of the scientific demystification of the plant *Kigelia africana,* it was noted that several studies showed a positive result for the many anecdotal medicinal properties of the plant, due to the many metabolites it contains. In the review he further highlights studies that looked at the chemical composition of the plant extract and such phytochemical groups as saponins, naphtoquines and flavanoids are present. Amandeep *et al,* (2013) conducted an analytical study that looked at the phytochemical, antioxidant and in vitro antibacterial activity of both the aqueous and ethanolic fruit extract of *Kigelia africana*. They aimed at assessing the levels of phytochemicals, enzymatic and non-enzymatic antioxidants and microbial activities. The results of the qualitative analysis showed that both ethanolic and aqueous extracts of *Kigelia africana* had alkaloids, glycosides, terpenoids, flavanoids tannins and reducing sugars. However the ethanolic extract did not test positive for saponins, this could be because saponins are most likely to easily dissolve in an aqueous extract than in an ethanolic extract. The methodology used in the part of the experiment that dealt with phytochemical analysis is similar to the one which will be used in this experiment except that this experiment shall not compare the photochemistry of the aqueous to the ethanolic extract. However, both water and alcohol, will be used for extraction in this experiment. The above mentioned study suggests that an aqueous solution has a better phytochemical composition than an ethanolic one, though it is expected that an extract done using alchohol will gather more organic compounds since 'like draws like' and also since lower temperatures of extraction are used when using organic solvents.

An analytical study conducted by Kumar *et al., (2012)* evaluated the anti-diabetic and hypolipidemic effect of *K. pinnata* flowers in Streptozotocin induced Wister rats. The results of this experiment showed a dose dependent blood sugar reduction in diabetic mice as well as a lipid lowering effect in the same mice. However, their method of induction does not give a specific diabetic type model even though the results showed a clear reduction in blood glucose levels and lipids after induction of diabetes was achieved. The reduction in comparison to the standard is not so low but is significant to provide a positive blood glucose acknowledgement. This method was not only able to give a clear picture of the changes in blood glucose but also showed the ideal changes in blood sugar when a standard is used and when no treatment is given. This experiment does not provide information concerning toxicity of the plant however; it gives a hint to answer the important question concerning toxicity, since at such a high dose of flower extract treatment none of the rats died. It would be interesting though, to see if a much higher dose than that used in the experiment would give better blood glucose lowering results and still be non-toxic to the animals.

Nyarko *et al,* (2005) conducted a comparative study of a herbal preparation ADD-199 and two oral hypoglycemic drugs for anti-diabetic activities in mice. ADD-199 is a herbal preparation that was made from *Maytenus senegalensis*, *Annona senegalensis*, *Kigelia africana* and *Lannea welwitschii*, which is traditionally prepared by west African traditional healers for diabetes. The anti-diabetic activity was investigated in streptozotocin induced mice. The study showed that the plasma glucose levels of the mice were reduced after being treated with ADD-199 in less than 2 weeks. Also insulin levels were increased in the diabetic mice by 70%. These results could be comparable to the maximum therapeutic doses of both glibenclamide and metformin. The results given by ADD-199 in this study could have been quite impressive because of the additive effect from several plants that exhibit anti-diabetic properties. An analysis of each single component would also be important to give as the individual plant's anti-diabetic activity. This study also gives us a hint as to how the herbs contained in the herbal preparation work especially when results are compared to the two conventional medicines that were used as standard, a study such as the one conducted by Nyarko *and Colleagues*, does also give a glimpse of what the mechanism of action of the plants used in the preparation ADD-199 is. However unlike Kumar *et al*, Nyarko *et al* in a different study though, also looked at toxicology of the herbal preparation

ADD-199in a Wister albino rats. They used two different doses of 100mg/kg and 500mg/kg of the extract and used hematological, urine and other biochemical parameters as well as modulations of some hepatic cytochrome P450 (CYP 450) isoenzymes. The results showed that alanine aminotransferase, alakaline phosphatse, aspartate aminotransferase and creatinine kinase levels were not affected. There was also no effect on the hematology though there was a dose dependant reduction of white blood cells by day 15 and recovery occurred by day 30 of treatment. The study concluded that no organ toxicity occurred and no potential drug interactions with drugs metabolized by CYP450 could occur. Even though this study gives a stepping stone in the direction of toxicology studies of these herbal medicines used to treat diabetes, it would be significant and give clarity if a study of the toxicology profile of each component of the herbal preparation was looked at. This is because ADD-199 is a preparation usually made by traditional practitioners, but most of the ordinary patients are likely to take one plant at a time.

Uhuo et al., (2014) conducted a comparative study of the effect of the stem and bark of the Nigerian plant on some biochemical parameters in diabetic induced rats. Similar to our study, they used Alloxan monohydrate to induce diabetes in the rats. The results of this experiment showed a drop in fasting blood sugar of mice that received the two different extracts in comparison to the Glibenclamide and the negative control. They also showed that the extracts increased the liver function tests of the rats and reduced the creatinine levels. From the study, the bark extract gave better results than the leaf extract. This study was a good starting point that showed the probable pharmacological activities and mechanisms of action of the extracts on the animals used. It was also good as it showed which part of the plant has better therapeutic activity. However, it would be a good point to also see if the fruit has better or less activity than the other parts of the plant. This study to a certain extent also showed that the plant may have some renal protective function. This is important in diabetes since some of the complications of diabetes are damage to the nephrons.

The study of *Kigelia africana* fruit provided a platform to understand it hypoglycemic potential. This study looked at anti-hyperglycemic properties of a part of the plant not studied in the studies mentioned above. Further, the study tackled the presence of any synergistic properties when the extract is used together with conventional medicine, Glibenclamide. A study of the fruit

chemistry provided a picture of comparison for the Zambian *Kigelia africana* and those that have been published concerning the tree growing in parts of the world.

CHAPTER THREE

3.0 METHODOLOGY

In this section, the methodology based on the study objectives will be discussed. It includes the research design, sampling technique, tools and reagents as well as data collection procedures and analysis.

3.1. Research design

This was an analytical study, a scientific experiment aimed at analyzing the effect of *K. africana* fruit extract on blood glucose as well as identification of basic phytochemical composition in order to give a base for future chemistry studies.

3.2. Chemicals and apparatus

Chemicals were obtained commercially and are of analytical grade. These included; Glibenclamide (Cipla), Alloxan monohydrate (sigma), Solvent (distilled water or methanol and dichlomethane) 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.

3.3.Botanical identification of the fruit

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. The fruit was harvested just before extraction in the months of December and January and used fresh.

Figure 1*;* *Kigelia fruit on the tree*

3.4. Ethical considerations

The study was approved by ERES converge IRB (Ethics Research and Science Converge). Being a purely laboratory-based experimental study involving the use of in vivo whole animal models, the ethical considerations were that this was a study that involved partial destruction of the pancreas in order to induce diabetes in mice models for purposes of testing the effectiveness of the extract. All scientific procedures and experiments conducted on the animal models were ethically approved. Requirements of good animal welfare and husbandry were strictly adhered to throughout the study. The animals after the study were disposed off in the most human way, which involved putting them in a desiccator with chloroform.

3.5.Study Subjects and Sampling Technique

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) and for easy handling, 15 mice were handled at a time.

3.6.Collection of fruit extract

The mature fruit was collected in the months of December and January. The fresh fruit was cut into pieces and put into a blender for a fine consistency. Extraction was done by two methods depending on the solvent.

a. About 100g of fruit was put into a large beaker and 150 mls of distilled water was added to collect the first extract. A series of five (5) extractions were done from the same fruit with the second through to the fifth extraction using 100mls. For extraction, the fruit was heated and brought to the boil. The mixture boiled for about 5 minutes before it was allowed to cool and then using a Buchner's funnel, it was filtered using suction filtration. The liquid extract is what was used for treatment of diabetes induced in mice models. Figure 1 shows the 5 series of extract.

Figure 2; *Series of fruit extract*

b. For the organic extract, about 10.19 g of fruit was used, enough solvent about 50 mls which is (dichloromethane and methanol in the ratio 1:1) to cover the fruit was put in a conical flask. The method used was maceration. The mixture stood with a mixer for 24 hours before it was also filtered using the Buchner's funnel and later left to dry.

The crude extract was dried in an oven at 40^0C and weighed before use in the mice. Only the first extract was used for treatment since after phytochemical analysis, it was seen that it had the most concentration of phytochemicals.

3.7.Phytochemical screening and Thin Layer Chromatography (TLC) of crude fruit extract

Following extraction, two methods of phytochemical analysis were done. The one involved thin layer chromatography (TLC) and the other involved analysis using reagents in test tubes.

3.7.1. Test for Tannins.

About 3 drops of 10% $FeCl_3$ was added to 2mls of fruit extract. A dark - blue precipitate was observed showing the presence of tannins.

3.7.2. Test for Saponins

About 5mls of crude extract was shaken vigorously with 5mls of distilled water in a test tube and warmed. A stable foam shows presence of saponins.

3.7.3. 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	Flavanoids
Orange color	Flavons
Red-violaceous color	Flavanones
Green	Flavanols

Because the original fruit extract was red in colour and the result of the above test also gives a red color, it was quite difficult to determine if the reaction is positive or not.

3.7.4. 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.

3.7.5. Steroid test

About 1ml extract was dissolved in 3mls of chloroform and then filtered. To the filtrate, concentrated Sulphuric acid [$H_2SO_{4\ conc}$] was added to form a lower layer. A redish brown ring was noted to show the presence of steroids.

3.7.6. Glycoside test

To 1ml of fruit extract, about 3mls glacial acetic acid containing 1 drop of 1% $FeCl_3$ (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.

3.7.7. Thin Layer Chromatography (TLC) of water and organic fruit extract of Kigelia africana

On a silica gel plate, with the aid of a micropipette, drops of the different series of fruit extracts were placed about 10-15 mm from the bottom of the plate and about 5-10 mm apart. The plate was allowed to dry up before being put in a vertical position into the chromatography chamber containing the solvent, which is the mobile phase of the chromatography. The TLC was run in a solvent combination of chloroform, methanol and water in a ratio of 9:6:1. After that, the solvent was allowed to evaporate at room temperature. A dry plate was later developed using a solution of 5% vanillin in methanol and two to three drops of concentrated Sulphuric acid.

A refractive index value was later calculated for the various stains seen on the TLC.

Rf = a/c where; *a* = the distance between the point of application and the centre of the spot of the material being examined and *c* = the distance between the point of application and the end of the mobile phase.

3.8.TEST OF KIGELIA FRUIT EXTRACT ON BLOOD GLUCOSE OF DIABETIC INDUCED MICE

3.8.1. Inducing diabetes in the mice and treatment

The mice were kept in a place that was well ventilated, had unrestricted access to food, water throughout the study. The mice were fed a usual diet which comprises 45% fat, 35% protein and 30% carbohydrate 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. There is no appropriate dose of extract in literature therefore; two doses were used on the mice based on other studies conducted. The mice had access to sunlight and experienced the usual cycle of about 12 hours of light and 12 hours of darkness.

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 after the 24 hour fasting period, before the Alloxan injection. Diabetes was induced in all mice using Alloxan monohydrate 90mg/kg intraperitoneal. Blood samples for random blood sugar were collected 72 hours after induction and used as a baseline. Treatment was started when mice had blood glucose of above 9.0mmol/l (Srinivasan, 2005). Treatment was given per oral once daily, using feeding needles at the same time according to the desired dosage for each product used. Blood samples for 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 will be 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.

Group 5- Mice treated with normal saline PO once daily.

Figure 3*; Group 1 mice in their housing cage*

To calculate the dose volume of Alloxan used for induction of diabetes the following formula was used; Dose volume = dose of Alloxan/1000 x weight of mouse/concentration of Alloxan

Figure 4*;* *feeding mice KAFE using feeding needle*

3.8.2. Blood collection for data collection procedure

In order to collect blood from the mice, the tail vein was punctured. This type of blood collection did not require one to kill the animal and hence was most suitable in this case. Mice were restrained and the tail is exposed. Topical anaesthesia (lidocaine gel) was applied on the lateral tail of the mouse**.** Using a lancet the tail of the mouse was pricked and a drop of blood sample was smeared on the ACCUCHECK glucose stick. The ACCUCHECK glucometer was used to give a blood glucose reading. Blood was collected from the mice every second day for 15 days.

Induced mice received treatment 72 hours post Alloxan monohydrate injection. The extract was diluted to concentrations 150mg/ml for treatment, while Glibenclamide was diluted to a concentration of 0.1 mg/ml. The dose volume was calculated using the following formula;

Dose volume = dose of drug/1000 x weight of mouse/concentration of drug

3.9. DATA ANALYSIS

A mouse was considered diabetic if blood glucose was above or equal to 9 mmol/l. A successfully treated mouse was considered to have blood glucose below 8 mmol/l but above 3.5 mmol/l. (Serreze *et al.,* 2000) A blood sugar of less than 3.0 mmol/l showed hypoglycemia.

Blood glucose results for each group of mice were expressed as a Mean +/- Standard deviation. An independent t- test used to analyze data; this is because data from two different groups was being compared at a time. The phytochemical analysis was descriptive and presented as present or absent.

CHAPTER FOUR

4. RESULTS

4.1. PHYTOCHEMICAL ANALYSIS

4.1.1. **Tannins**; A dark blue precipitate was observed for the presence of Tannins. However, the concentration reduced with the increase in the number of extractions from the same product as shown in the picture below;

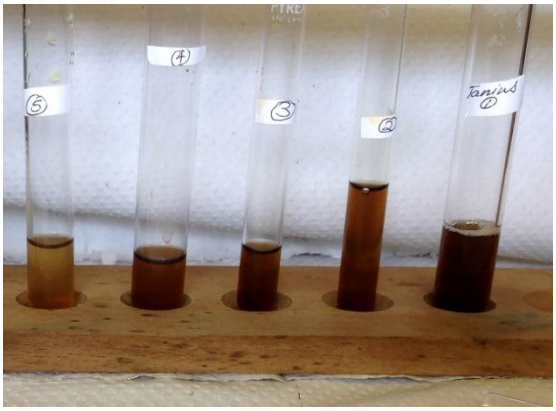

Figure 5; *Dark blue precipitate, seen with decreasing intensity from test tube 1 though to test tube 5*

4.1.2. **Saponins**; A steady foam was observed on top of the fruit extract after vigorous shaking. The intensity in this case also reduced as the series of extraction increased.

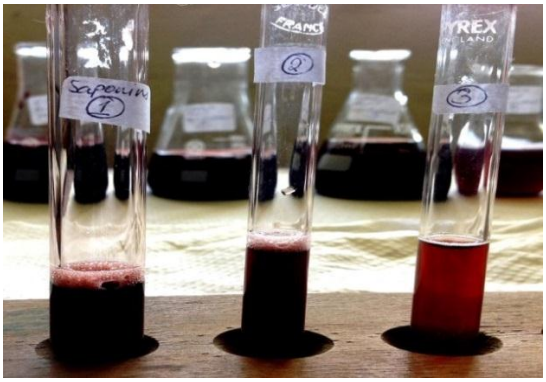

Figure 6; *Steady foam seen above the fruit extract with decreasing intensity showing the presence of saponins*

4.1.3. **<u>Flavanoids;</u>** a red colour was observed with decreasing intensity as the series of extraction increased.

Figure 7; *Positive results for flavonoids, a red colour with decreasing intensity from test tube 1 to test tube 5*

4.1.4. **<u>Alkaloids</u>**; A dark precipitate was observed confirming the presence of alkaloids**<u>.</u>** However intensity reduced with increase in series of extraction.

Figure 8; *Precipitate seen in the test tubes showing the presence of alkaloids with decreasing intensity*

4.1.5. **Steroid test;** A reddish-brown ring was observed to prove the presence of steroids. This test was conducted on the organic extract only.

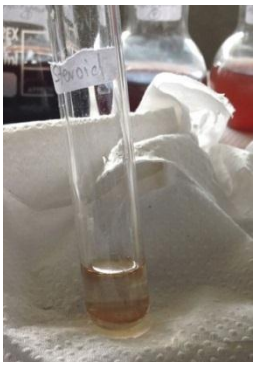

Figure 9; *A redish- brownish ring to confirm the presence of steroids*

4.1.6. **Glycoside test**; A green layer was observed showing the presence of glycosides. This test was conducted on the organic extract only.

Figure 10; *Green layer to show the presence of glycosides*

The above mentioned tests were done on all the 5 series of extracts, except for the test for steroids and glycoside that were conducted on the organic extract. The table below presents the results for all the tests done on the series of extraction.

Table 2; results on phytochemical analysis of extract series

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

From the above results, the fruit extract showed a positive result for the presence of alkaloids, saponins, tannins, flavonoids, steroids and glycosides. The first 3 extractions gave clear positives while the last two either had negative or a somewhat positive result. 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 tests require a dry product and the organic extracts dried the quickest.

4.1.7. Thin Layer Chromatography (TLC) of water and organic fruit extract of Kigelia Africana

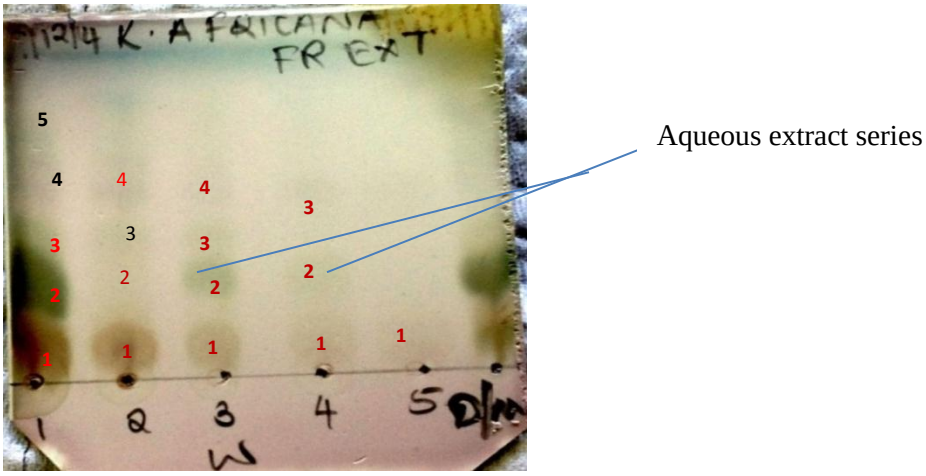

Figure 11; *Thin Layer Chromatography of the 5 series of aqueous fruit extract*

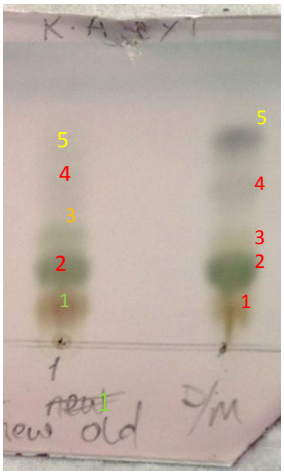

Figure 12; *TLC comparing aqueous extract 1 with organic extract*

Figure 11 shows a TLC for the five (5) aqueous series of fruit extract and how they behaved. In the first series we see 5 spots present with spots 1, 2 and 3 showing a high concentration. As the series increases concentration of the phytochemicals seen in the 1st series reduces and some spots are even absent in the fourth (4th) and fifth (5th) series. This is in agreement with the test tube experimental results seen.

The tables below show the Refractive index values (Rf) values in mobile phase chloroform, methanol and water (9:6:1) of the aqueous series (Table 2) and the comparison of the aqueous and organic extract (Table 3). From the results presented in Table 3, we can see that similar phytochemicals are extracted using both aqueous and organic extract. This is shown by the Rf values that are very similar to eachother.

Table 3; Rf values for figure 11 TLC of aqueous extract series

Spot #/ test ID #	**1**	**2**	**3**	**4**	**5**
1	0.10	0.10	0.10	0.10	0.10
2	0.27	0.27	0.29	0.30	0.31
3	0.47	0.45	0.43	0.59	0.60
4	0.62	0.60	0.60		
5	0.70				

Table 4; Rf values for figure 12 TLC of aqueous extract and organic extract

Spot #/ test ID #	**Aqueous extract**	**Organic extract**
1	0.10	0.11
2	0.23	0.26
3	0.36	0.37
4	0.52	0.52
5	0.63	0.65

4.2. EFFECT OF KIGELIA AFRICANA FRUIT EXTRACT ON BLOOD GLUCOSE

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 as shown in figure 14. 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 16. 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 15.

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 13. The following tables show the average blood glucose in the five groups of mice. The readings indicate; fasting blood glucose that was taken before induction of diabetes with Alloxan monohydrate (Table 5); random blood glucose 72 hrs after Alloxan monohydrate but before treatment (Table 6); and random blood glucose readings after 14 days of treatment (Table 7). The figures 13-16 show a graphic presentation of the blood glucose among different groups.

Table 5; Average fasting blood glucose (FBG) of mice in five different groups

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
Std. Deviation	+/-0.8mmol/l	+/-1.9mmol/l	+/-1.2mmol/l	+/-0.9mmol/l	+/-1.0mmol/l

KAFE* - Kigelia Africana Fruit Extract

Table 6; Average Random blood glucose (RBG) 72 hours after Alloxan injection

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
Std.Deviation	+/-1.9mmol/l	+/- 1.1mmol/l	+/- 3.6mmol/l	+/- 2.7mmol/l	+/- 3.6mmol/l

KAFE* - Kigelia Africana Fruit Extract

Table 7; Average random blood glucose (RBG) reading with standard deviations on day 15 after treatment

Group ID	KAFE* 1000mg/kg	KAFE* 500 mg/kg	Glibenclamide 0.25 mg/kg	Glib +KAFE* 500 mg/kg	Normal Saline
Mean RBG +/- SD	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

*KAFE- Kigelia africana fruit extract, Glib- Glibenclamide

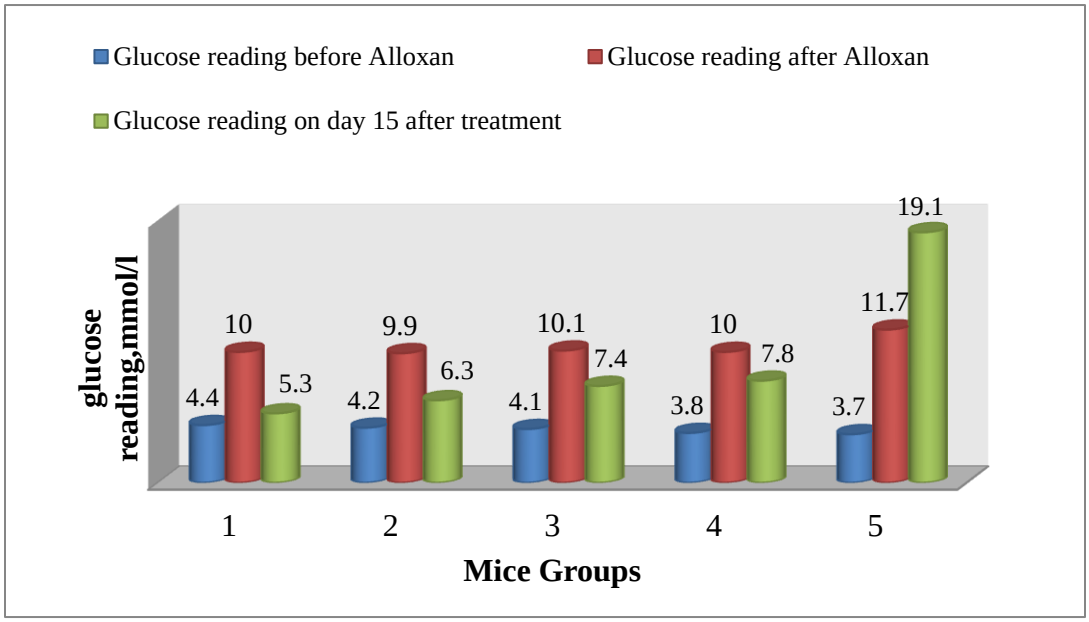

Figure 23; *Summary of blood glucose readings before and after treatment*

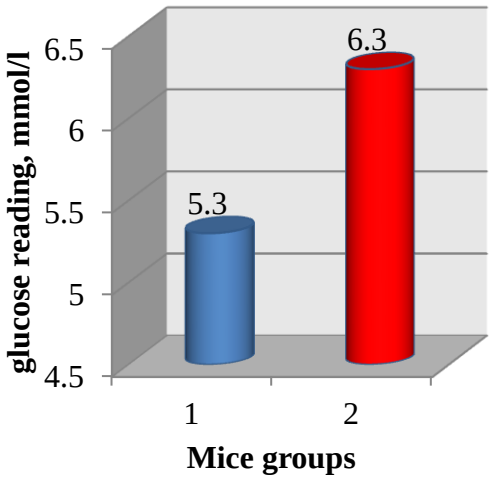

Figure 14; *Dose dependent reduction seen with KAFE 1000mg/kg vs 500mg/kg,[(5.3+/- 0.5 mmol/l vs (6.3+/- 0.6 mmol/l) p= 0.005]*

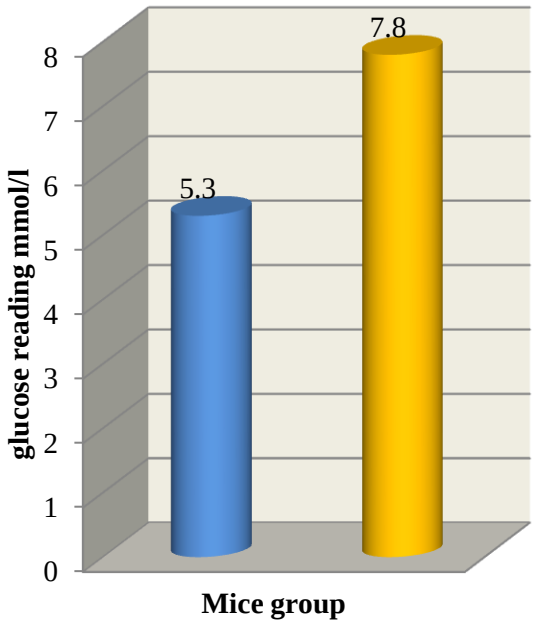

Figure 15; *Poor blood reduction in concomitant use than single high dose use,[(7.8+/- 0.6mmol/l vs 5.3 +/- 0.5mmol/l) p=0.000]*

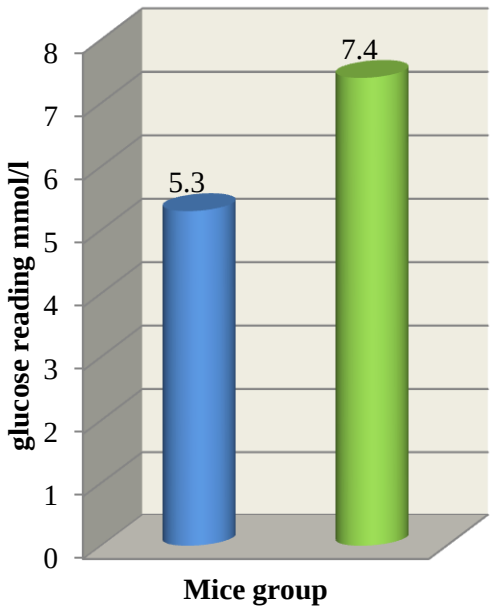

Figure 16; *Poor blood reduction with Glibenclamide than with KAFE 1000mg, [(5.3 +/- 0.5 mmol/l vs 7.4 +/- 0.9 mmol/l) p=0.00]*

CHAPTER FIVE

5.0 DISCUSSION

This discussion is based on the results of the first and second specific objectives. The key findings are that; the fruit extract shows the presence of phytochemicals namely, alkaloids, tannins, saponins, flavonoids, steroids and glycosides. The study also showed that the fruit extract has a dose dependent reduction in the random blood glucose level of mice induced with diabetes ($p=0.02$), fig 14. Further, the study shows a significant reduction in blood glucose of mice induced with diabetes that received Kigelia extract 1000mg/kg in comparison to mice that received a combination of Glibenclamide 0.25mg/kg and Kigelia extract 1000mg/kg ($p=0,00$) fig 15.

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 extraction was conducted, positivity reduced with the increase in series. This was seen on the TLC conducted showing an absence of certain spots (spot 4 and 5 totally disappearing) with the rise in series of extraction; fig 11. Through the TLC it was also found that the concentration of extract was higher in the organic extract than in the aqueous extract. This is shown by the reduction in concentration of the same spots seen in the organic TLC as compared to the aqueous TLC, fig 12. Olatunji and Colleagues' review as well as Amandeep and Colleagues 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 collegues mention that there is a strong association between the hypoglycemic properties of the plant and the presence of alkaloids and it was thought 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 (BNF, 2009). 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.

One of the questions addressed by the present study was how Kigelia africana fruit extract affects blood glucose of diabetic induced mice. The 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^{th} day of treatment there was a statistically significant reduction in the average RBG level of mice on KAFE 1000 mg/kg is 5.3+/-0.5 mmol/l while mice on KAFE 500mg/kg is 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 (2012) even though their study was conducted in rats that were induced with streptozotocin and also, flowers instead of the fruit were used. Further, Uhuo and Colleagues (2014) 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, Uhuo and Colleagues used rats though induced by Alloxan monohydrate like in this study. The reduction in the fasting blood glucose of rats conducted by Uhuo and colleages 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.

Another question the study addressed was 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.15). Glibenclamide has insulin secretogogical properties on residual beta cells of the pancreas, (BNF, 2009). 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 (Nyarko, 2005). 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 the high dose of fruit extract alone, the lower dose of fruit extract alone and 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 it 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.

CHAPTER SIX

6.0. CONCLUSIONS AND RECOMMENDATIONS

6.1 Study limitations

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.

6.2. Conclusion

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

6.3. Recommendation

There is a need for isolation of compounds and to identify the compounds with antidiabetic properties, in addition the mechanism of action of the extract needs to be established as this will help establish possible pharmacological interactions.

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Appendix SPSS data

Descriptive Statistics before induction of diabetes (ref Table 5)

	N	Minimum	Maximum	Mean	Std. Deviation
KAFE 1000mg/kg	7	3.5	5.4	4.371	.6343
KAFE 500mg/kg	7	2.7	5.9	4.200	1.1776
Glib 0.25mg/kg	7	2.0	5.4	3.800	1.1916
Glib and KAFE	7	2.3	5.2	3.943	.9710
Normal Saline	7	2.9	6.0	4.014	1.0479
Valid N (listwise)	7				

Descriptive Statistics after induction with Alloxan monohydrate (ref Table 6)

	N	Minimum	Maximum	Mean	Std. Deviation
KAFE 1000mg/kg	8	9.00	10.00	9.5500	.34226
KAFE 500mg/kg	8	8.70	13.80	10.2375	1.82125
Glib 0.25mg/kg	7	8.40	15.10	10.3429	2.21123
Glib and KAFE	7	8.40	22.30	10.7857	5.09720
Normal Saline	7	8.70	15.00	11.2714	2.35352
Valid N (listwise)	7				

Descriptive Statistics on day 14 after treatment (ref Table 7)

	N	Minimum	Maximum	Mean	Std. Deviation
7KAFE 1000mg/kg	7	4.5	6.0	5.271	.5469
7KAFE 500mg/kg	7	5.3	7.0	6.314	.5757
7Glib 0.25mg/kg	7	5.9	8.9	7.443	.9396
7Glib and KAFE	7	7.0	9.0	7.829	.6448
7Normal Saline	7	10.4	30.0	19.186	8.4626
Valid N (listwise)	7				

T-Test – Group 1 and 2 (figure 14)

Group Statistics

	group	N	Mean	Std. Deviation	Std. Error Mean
sugar	k1000	7	5.271	.5469	.2067
	k500	7	6.314	.5757	.2176

Independent Samples Test

		t-test for Equality of Means						
		t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
							Lower	Upper
sugar	Equal variances assumed	-3.475	12	.005	-1.0429	.3001	-1.6967	-.3890
	Equal variances not assumed	-3.475	11.968	.005	-1.0429	.3001	-1.6969	-.3888

T-Test – Group 1and 3 (figure 15)

Group Statistics

	group	N	Mean	Std. Deviation	Std. Error Mean
sugar	k1000	7	5.271	.5469	.2067
	g0.25	7	7.443	.9396	.3551

Independent Samples Test

		t-test for Equality of Means						
		t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
							Lower	Upper
sugar	Equal variances assumed	-5.284	12	.000	-2.1714	.4109	-3.0667	-1.2761
	Equal variances not assumed	-5.284	9.646	.000	-2.1714	.4109	-3.0916	-1.2513

T-Test – group 1 and 4 (figure 16)

Table 14- Group Statistics

	group	N	Mean	Std. Deviation	Std. Error Mean
sugar	k1000	7	5.271	.5469	.2067
	g/k	7	7.829	.6448	.2437

		t-test for Equality of Means						
		t	Df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
							Lower	Upper
sugar	Equal variances assumed	-8.002	12	.000	-2.5571	.3195	-3.2534	-1.8609
	Equal variances not assumed	-8.002	11.689	.000	-2.5571	.3195	-3.2554	-1.8589

Induction of diabetes using alloxan monohydrate and Treatment of mice with Kigelia fruit extract

dose 1
500mg/kg and
dose 2
1000mg/kg

Dosages required is 90mg/kg

Dosage of solution if 0.25%

Meaning: 250mg of alloxan mono hydrate in 10 mls of normal saline.

Dose volume calculation for each mouse;

dose /1000 * weight of animal/concentration of solution

90/1000*20/25= 0.072ml

Table 1- dose volumes for Alloxan and oral interventions.

Mouse Identification	ip dose vol	po dose vol	kg mouse
group 1- KAFE 1000mg/kg			
group 2- KAFE 500mg/kg			
group 3- Glibenclamide 0.25 mg/kg			
group 4 – Glibenclamide + KAFE 500 mg/kg			

group 5 Normal saline			

Table 2- blood glucose before and after intervention.

Mouse Identification	fbs	rbs after alo	rbs 1	rbs 2	rbs 3	rbs 4	rbs 5	rbs 6	rbs 7
group 1									
group 2									
group 3									
group 4									

group 5 Normal saline			

Table 2- blood glucose before and after intervention.

Mouse Identification	fbs	rbs after alo	rbs 1	rbs 2	rbs 3	rbs 4	rbs 5	rbs 6	rbs 7
group 1									
group 2									
group 3									
group 4									

group 5									

Table 3 of mass of crude extract

Serial #	Volume of solvent used	Volume of crude extract after extraction	Mass of crude extract
1.			
2.			
3.			
4.			
5.			
Organic extract			

Table 4- Test for presence of phytochemical compounds

Test	1	2	3	4	5
Tannins					
Saponins					
Flavanoids					
Alkaloids					
Steroid test for organic extract					
Glycoside test for organic extract					

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I.R.B. No. 00005948
F.W.A. No. 00011697

12th November, 2014

Ref. No. 2014-Sept-013

The Principal Investigator
Ms. Tumelo Muyenga
University Teaching Hospital
Dept. of Pharmacy
P/Bag RW 1X,
LUSAKA

Dear Ms. Muyenga,

RE: EFFECT OF KIGELIA AFRICANA FRUIT EXTRACT ON BLOOD GLUCOSE IN DIABETIC INDUCED MICE.

Reference is made to your corrections dated 13th November, 2014. The IRB resolved to approve this study and your participation as principal investigator for a period of one year.

Review Type	Ordinary	Approval No. **2014-Sept-013**
Approval and Expiry Date	Approval Date: 12th November, 2014	Expiry Date: 11th November, 2015
Protocol Version and Date	Version-Nil	11th November, 2015
Information Sheet, Consent Forms and Dates	• N/A	11th November, 2015
Consent form ID and Date	Version-Nil	11th November, 2015
Recruitment Materials	Nil	11th November, 2015
Other Study Documents	N/A	11th November, 2015
Number of participants approved for study	-	11th November, 2015

Specific conditions will apply to this approval. As Principal Investigator it is your responsibility to ensure that the contents of this letter are adhered to. If these are not adhered to, the approval may be suspended. Should the study be suspended, study sponsors and other regulatory authorities will be informed.

Conditions of Approval

- No participant may be involved in any study procedure prior to the study approval or after the expiration date.
- All unanticipated or Serious Adverse Events (SAEs) must be reported to the IRB within 5 days.
- All protocol modifications must be IRB approved prior to implementation unless they are intended to reduce risk (but must still be reported for approval). Modifications will include any change of investigator/s or site address.
- All protocol deviations must be reported to the IRB within 5 working days.
- All recruitment materials must be approved by the IRB prior to being used.
- Principal investigators are responsible for initiating Continuing Review proceedings. Documents must be received by the IRB at least 30 days before the expiry date. This is for the purpose of facilitating the review process. Any documents received less than 30 days before expiry will be labelled "late submissions" and will incur a penalty.
- Every 6 (six) months a progress report form supplied by ERES IRB must be filled in and submitted to us.
- ERES Converge IRB does not "stamp" approval letters, consent forms or study documents unless requested for in writing. This is because the approval letter clearly indicates the documents approved by the IRB as well as other elements and conditions of approval.

Should you have any questions regarding anything indicated in this letter, please do not hesitate to get in touch with us at the above indicated address.

On behalf of ERES Converge IRB, we would like to wish you all the success as you carry out your study.

Yours faithfully,
ERES CONVERGE IRB

Dr. E. Munalula-Nkandu
BSc (Hons), MSc, MA Bioethics, PgD R/Ethics, PhD
CHAIRPERSON

THE UNIVERSITY OF ZAMBIA

DEPARTMENT OF BIOLOGICAL SCIENCES

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Fax: +260-211-253952

P.O. Box 32379
Lusaka
Zambia

Your ref:
Our ref:

IDENTIFICATION RESULTS

Date: **10th July 2014**

Name of specimen (s):

1. ***Kigelia african*** **(Lam.) Benth.**

Number of specimens: **1**.....

Identified by: **Florence C. Nyirenda (MSc.)**

Designation: **Herbarium Assistant / Senior Technician**

Signature: .....

THE UNIVERSITY OF ZAMBIA
SCHOOL OF MEDICINE

DEPARTMENT OF PHARMACY

INTERNAL MEMORANDUM

To: The Head, Physiological Sciences,

School of Medicine, UNZA

From: Acting Head, Department of Pharmacy

Date: 22th August, 2014

Subject: Tumelo Muyenga

Kindly note that the above named is a student in the Department of Pharmacy currently pursuing her Masters' in Clinical Pharmacy.

The topic for her research is," THE EFFECT OF KIGELIA AFRICANA FRUIT EXTRACT ON BLOOD GLUCOSE IN DIABETIC INDUCED MICE," which she is pursuing for the award of the Master's degree in Clinical Pharmacy.

Tumelo will require using the facilities in the Biochemistry and Pharmacology Laboratory at Ridgeway campus under Physiological Sciences.

I write this memorandum seeking your permission to allow her to use the facilities.

Thanking You,

Dr Prashar

Ag. HOD, Department of Pharmacy

School of Medicine, UNZA

THE UNIVERSITY OF ZAMBIA
SCHOOL OF MEDICINE
HEAD OF DEPARTMENT
22 SEP 2014
DEPARTMENT OF
PHYSIOLOGICAL SCIENCES