KAMPALA INTERNATIONAL UNIVERSITY

WESTERN CAMPUS

INVESTIGATION OF ANTI-HYPERGLYCEAMIC PROPERTIES OF THE AQUEOUS EXTRACT OF GREEN PEEL OF *MUSA ACUMINATA* *'NAKITEMBE CULTIVAR'*

BY

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DEDICATION

This research work is dedicated to all diabetic patients, my family and classmates.

DECLARATION

I hereby declare that this research dissertation titled "Investigation of anti-hyperglyceamic properties of the aqueous extract green peel of *musa acuminata ' nakitembe cultivar* 'is genuine research work to be carried out by me under the guidance of Mr. Nnamdi Ejekwamadu

Signature.....

Mwesigwa Wilson

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APPROVAL

I certify that this work is original research carried out by Mwesigwa Wilson of the School of Pharmacy, Kampala International University-Western campus, Ishaka -Bushenyi, Uganda.

..... Date: .....

Mr. Nnamdi Ejekwamadu

SUPERVISIOR

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I would like to thank God for the wisdom, guidance and mental stability throughout my study.

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ABBREVIATIONS

DM	Diabetes Mellitus
WHO	World Health Organization
spp.	Species
IDF	International Diabetic Federation
NS	Normal saline
BS	Blood sugar

ABSTRACT

The objective of this research was to investigate the anti-hyperglyceamic properties of the aqueous extract green peel of (Banana) *musa acuminata 'nakitembe cultivar'* in alloxan induced diabetic rats. The extract used was obtaned from the green banana dried and phytochemical analysis and acute toxicity study carried out.

Alloxan was used to induce diabetes in experimental albino Rats at a dose of 140mg/kg body weight. After three days, the animals blood sugar levels was tested and those with BS above 7mmol/l were selected for the study. One group was the normal control with normal BS levels.

Goup I was normal control treated with normal saline, Group II was diabetic control treated with normal saline 2ml/kg body weight. Group III and IV recieved extract at a dose of 400 and 800 mg/kg body weight. Group V was treated with standard drug insulin at a dose of 0.03IU/kg body weight. The animals were treated daily and BS was determined every after three days. The project was carried out for 15 days. Blood for testing BS levels was obtained from tail vein.

Results ; Phytochemical analysis revealed presence of saponins, tannins, phenols, flavonoids, cardiac glycoside, alkaloids, steroids and terpenoids. The extract showed no signs of toxicity such as salivation, diarrhoea, depression, stimulation, vomiting, and comma and there was no mortality observed up to a dose of 4000mg/kg. Administration of the extract to GROUP III and GROUP IV did not show any significant changes blood sugar levels as compared to that of GROUP V which received the standard drug insulin.

Conclusion; At the doses investigated, the aqueous extract of the green banana peel did not possess significant anti-hyperglycemic effect. However it contains phytochemicals which have antioxidant properties useful in preventing complication brought about by glucose toxicity in diabetic patients. The flavonoid content possess significant effect on normalization of serum creatinine level and lowering of TG and relative weight of liver, indicating possible presence of kidney and liver protective property. The green banana peels have an added advantage thus diabetic patients should continue boiling the banana with the peels.

CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND

347 million people worldwide have diabetes mellitus(the silent killer) WHO (2013). Africa is experiencing a rapid epidemiological transition with the burden of non communicable diseases especially diabetes that will overwhelm the health care systems which is already overburdened by HIV/AIDS, TB and Malaria. This is due to rapid urbanization and westernization of lifestyle, rapidly decreasing physical activity, changes in dietary habits, ageing of the population.

In Uganda Prevalence of diabetes in adults (20-79 years) 4.14% and total new cases in 2013 were 625 (International Diabetic Federation, 2013)

1.1.1 Diabetes mellitus

Diabetes is a chronic metabolic disease that occurs when the human body is not able to produce enough of the hormone insulin or because cells do not respond to the insulin that is produced. High blood sugar produces symptoms of frequent urination, increased thirst and hunger.

1.1.2 Types of DM

There are two types of diabetes, namely;

Insulin dependent Diabetes mellitus (IDDM) type I and Non-Insulin Dependent Diabetes mellitus(NDDM) type II. The type I occurs in young people, usually below 35 years of age; while the type II occur in older people usually above 35 years old. Weight is a predisposing factor. In type I, the pancreas cannot make insulin, so the patient must be treated with insulin in the absence of which they cannot survive. The patient receives insulin injections once or twice a day.

On the other hand, in type II, the pancreas does produce insulin, but the body cannot use the insulin properly. In this case, the patient is treated with oral medication. Approximately 80-90% of diabetes is type II. Insulin resistance is strong factor.

Gestational diabetes occurs when pregnant women without a previous diagnosis of diabetes develop a high blood glucose level; it can lead to serious risks to the mothers and her infant and increases the risk for developing type 2 diabetes later in life.

Recent studies suggest that, the recorded prevalence of diabetic retinopathy, neuropathy, microalbuminuria (nephropathy) have increased. Several reviews have reported the common occurrence of gangrene, infection and sepsis associated with diabetic foot ulcer disease in Sub-Saharan Africa. About 12% of all diabetic patients have foot ulcers, and amputations occur in up to 7% of all hospitalised diabetic patients. (*Hall et al 2011*).

There's is no effective therapy yet to treat DM though insulin and biguanide and sulphonylurea therapy are used. There's high incidence of side effects and resistance to drugs, thus the need for natural products with minimal side effects. Herbal drugs have been the major source for treatment of many ailments since antiquity in Africa and many other parts of the world. Herbal medicines have been the highly esteemed source of medicine and have become a growing part of modern, approaches of diabetes. In view of the above the present research would evaluate hypoglycemic properties of green banana peels.

Medicinal plants with hypoglycemic properties play an important role in reducing the complications of diabetes. In this aspect, the banana plant, a member of the musaceae family, is known to have hypoglycemic effects and was used to treat diabetes in traditional practices of medicine *(Joshi 2000)*.

1.1.3 Alloxan and induction of diabetes

In animals, it can be induced by partial pancreatectomy or by the administration of diabetogenic drugs such as alloxan, streptozotocin, ditizona and anti -insulin serum. These agents selectively destroy the Langerhans islet ß-cells. The best known drug-induced diabetes model is the alloxan diabetes. Alloxan, a derivative of uric acid, as well as of other substances of different chemical groups, causes ß-cell to degranulate and consequently degenerate (*Eliziane et al., 2003)*

Bananas are one of the most consumed fruits in tropical and subtropical regions. Bananas are tree like perennial herbs 2-9 meters in height. They are vegetatively propagated from the rhizome. In the

traditional medicinal systems of India, all the parts of *Musa spp.* (family *Musaceae*) are used for the treatment of various diseases. (*Gurumaa et al, 2008*). The peel of banana represents 40% of the total weight of the fruit. Peel contains potassium (K), calcium (Ca), sodium (Na), iron (Fe), manganese (Mn), Copper (Cu), bromine, rubidium, strontium, zirconium and niobium. Banana peel extracts has been proved to have antioxidant properties. Other parts of plant such as flower and the stem have anti hyperglycemic properties and very little knowledge is present about anitidiabetic properties of green peel despite banana being readily available to the people hence the present study.

Musa paradisiaca (banana) is known for its antidiabetic activity in animal mode. Different parts of plant like stem, flower, leaves, fruit roots have shown medicinal properties. Bananas and plantains constitute the fourth most important global food commodity (after rice, wheat and maize) grown in more than 100 countries over a harvested area of approximately 10 million hectares, with an annual production of 88 million tonnes (*Frison and Sharrock, 1999*). The all year round fruiting habit of bananas puts the crop in a superior position in bridging the 'hunger gap' between crop harvests

There are many composition of banana skin like enzymes such as *polyphenoloxidases, pectin* as a gelling agent and that banana peel extract alone or combined with cream or ointment. Medicinal benefits of the extract include relief of pain, swelling and itching (*Wuyts et al, 2006*). Additionally *flavonoids, tannins, phlobatannins, alkaloids, glycoside and terpenoids* are present in the peels of Musa family. These have been reported to have multiple biological and pharmacological effects (antibacterial, antihypertensive, antidiabetic and anti-inflammatory activities). The presence of these bioactive substances in banana peel therefore suggests that the peel possess valuable medicinal potential yet to be explored.

Banana peel also contains *vitamins (A, C, E, B6), Galloctechin, mallic acid, succinic acid, magnesium, phosphorus, fiber and iron*

The alarming rate at which traditional medicine is now patronized by all segments of the society- the rich, the poor, educated and the uneducated- clearly signifies one thing, "the realization that traditional medicine which has long been taken for granted and rejected for decades has a crucial role to play in making affordable health care delivery system available to the entire populace"

1.4 Objectives

1.4.1 General objective

To investigate the anti-hyperglyceamic properties of the aqueous extract green peel of *musa acuminata 'nakitembe cultivar'* in alloxan induced diabetic rats

1.4.2 Specific objectives

- To prepare an aqueous extract of green banana peel .
- To carry out phytochemical investigation on the extract.
- To determine the acute toxicity concentration of the extract.
- To estimate blood glucose levels of case and control population of rats.

1.5 Hypothesis

Null hypothesis

Green banana peel extract does not possesses anti-hyperglycemic activity

Alternative Hypothesis

Green banana peel extract possesses anti-hyperglycemic activity

(*Olapade, E. O, 1992*). As a result of increase demand for alternative medicine, renewed interest in drugs of plant origin has been growing steadily.

Although, much survey had been carried out on plants, more survey is still necessary with regard to the plants used in treating diabetes as there is increase in the rate of Diabetes mellitus manifestation in Uganda and African countries in general.

1.2 Statement of problem

No satisfactorily effective therapy is yet to cure diabetes mellitus. Though insulin therapy, glipuride and others are used for the management of diabetes mellitus, there are several drawbacks like insulin resistance, anorexia nervosa, brain atrophy and fatty liver. Requirement for refrigeration of the drug, skilled technicians and high of insulin per month, are not affordable in poor a economic community. Chronic treatment with sulfonylurea and biguanides are also associated with side effects.

Plant drugs are frequently considered to be less toxic and free from side effects than the synthetic ones.

1.3 Justification of the study

- Banana is a staple food in Uganda.
- Different parts of the banana plant have previously been demonstrated to have therapeutic value, including anti-hyperglycemic effect.
- In south-western Nigeria, green banana is believed to alleviate diabetes mellitus when boiled with the peelings (Ojewole & Adewunmi, 2003).
- In Uganda, little is known about this alleged medicinal property.
- Although banana peelings have been demonstrated to have anti-microbial activity, nformation is lacking on any possible anti-diabetic properties.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Background

An increasing amount of evidence shows that the consumption of fruits and vegetables is, in general, beneficial to health due to the protection provided by the antioxidant compounds contained in them. In fact, the presence of phytochemicals, in addition to vitamins and provitamins, has been considered of great nutritional interest in the prevention of chronic diseases, such as cancer, arteriosclerosis, nephritis, diabetes mellitus, rheumatism, ischemic and cardiovascular diseases and also in the aging process,

Taxonomical classification

Kingdom: *Plantae*

Division: *Magnoliophyta*

Class: *Liliopsida*

Order: *Zingiberales*

Family: *Musaceae*

Genus: *Musa*

Species: *Musa paradisiaca*

Banana is a familiar tropical fruit. From its native South western Pacific home, the banana plant spread to India by about *600 BC* and later on it spread all over the tropical world. It is possibly the world's oldest cultivated crop. It even spread into the Islands of the Pacific and to the West Coast of Africa as early as *200-300 BC* (*Rahman and Kabir, 2003*).

Musa paradisiaca is a herbaceous plant (up to 9 m long) with a robust treelike pseudo stem, a crown of large elongated oval deep-green leaves (up to 365 cm in length and 61 cm in width), with a prominent midrib, each plant produces a single inflorescence like drooping spike, and large bracts opening in succession, ovate, 15-20 cm long, concave, dark red in colour and somewhat

fleshy. Fruits are oblong, fleshy, 5-7cm long in wild form and longer in the cultivated varieties. *Musa sapientum* is a treelike perennial herb that grows 5 - 9 m in height, with tuberous rhizome, hard, long pseudo stem. The inflorescence is big with a reddish brown bract and is eaten as vegetables. The ripe fruits are sweet, juicy and full of seeds and the peel is thicker than other banana.

Musa spp (Musaceae), called banana in English, are one of the interesting tropical plants which have been consumed since centuries by humans and animals as a nutritious food. Yet, ethno botanists do not know exactly where the plant originated. The banana is such a pan-tropical that it grows everywhere man has planted it. There are hundreds of edible banana varieties;

Musa species (Musaceae), atropicalplant, have been consumed since many years by mankind for its nutritious and delicious fruits. In addition to this, Musa species have been reported to have various biological activities such as *antiulcerogenic, antidiabetic, antiatherogenic, antidiarrheic, antitumoral, antimutagenic, antidepressant* and have been also found to be effective in treatment of migraine, hypertension, cholesterol.

The peels of a variety of fruits have gained attention as a natural source of antioxidants and phytochemical content which are rich in compounds with free radical scavenging activity. Banana and Plantain peels are major agricultural wastes which have been used as medicine, animal feeds, blacking of leathers, soap making, fillers in rubber and so on *(Arawande et al, 2010).*

Musa paradisiaca (Banana) is a tree like herb (*Family: Musaceae*) with thick stem composed of convoluted leaf sheaths. Leaves are very oblong. It is distributed through out Central Africa, India and Malaysia and all over the world. And belong to musaceae family. Flower of Musa sapientum (another species of banana) showed hypoglycemic activity on normal fasting rabbits. The biological activities reported contained in the different parts of Musa paradisiaca are mainly the leaves, roots, fruits, stem and seed.

Chemically *Musa paradisiaca* contains starch (unripe pulp fruit,) iron, carbohydrates, flavonoids, diflavonoids, tannins, phenolic compounds etc

In folk medicine all parts of Musa paradisiaca are used to cure several disorders. Fruit peel of Musa paradisiaca has been used for its antimicrobial activity against *Bacillus subtilis, Staphylococcus aureus, Escheerichia coli, Pseudomonas aeruginosa, Candida albicans and Cryptococcus neoformans* in comparison with the standards benzyl penicillin and streptomycin (*Mangathayaru et al, 2004*)

Green banana peel extract at different concentrations can be used to treat depression (*Tan et al, 2011*)

In another study on banana (*Musa sapientum*), mainly used in Indian folk medicine for the treatment of diabetes mellitus, oral administration of chloroform extract of the banana flowers in alloxan-induced diabetic rats for 30 days resulted in a significant reduction in blood glucose, glycosylated heamoglobin and an increase in total haemoglobin. Oral glucose tolerance test was also performed in diabetic rats in which there was a significant improvement in glucose tolerance in animals treated with the banana flowers and the effect was compared with glibenclamide. (*Pari, et al)*

The green fruit of *M. paradisiaca has* been reported to have hypoglycemic effect due to stimulation of insulin production and glucose utilization (*Ojewole and Adewunmi, 2003).* Its high potassium (K) and sodium (Na) content has been correlated with the glycemic effect (*Rai et al., 2009*). Fibers from *M. paradisiaca* fruit increased glycogenesis in the liver and lowered fasting blood glucose. Antihyperglycemic effect of the hydromethanolic extract of M. paradisiaca root has been found significant (*Mallick et al., 2006; Mallick et al., 2007*). The chloroform extract of flowers of *M. sapientum* showed blood glucose and glycosylated haemoglobin reduction and total haemoglobin increase after oral administration in rats. It also controls lipid peroxidation in diabetes (*Pari and Maheshwari, 2000).* However, *M. paradisiaca* stem juice showed hyperglycemic activity (*Singh et al, 2007*). Isolated pectin from the juice of the inflorescence stalk of *M. sapientum* increases the glycogen synthesis and decreased glycogenolysis and gluconeogenesis.

CHAPTER THREE

3.0 METHOD AND MATERIALS

3.1 Study design

This study was experimental; involving alloxan induced diabetic as well as normal albino rats

3.2 Study Area

The sample was sourced from Bushenyi District and the study was carried out in the Pharmacognosy and Pharmacology Laboratories of KIUWC, Ishaka.

3.3 Study population

The study was conducted in mature experimental albino rats.

3.4 Sample size:

A total of 39 animals was used, in line with *(Campos et al ., 2013)*

9 albino rats were used for acute toxicity tests.

30 albino rats were used for the anti-hyperglycemic activity study

3.5 Sampling technique:

Random sampling technique was used.

3.6 Inclusion criteria

Only mature, healthy adult male and female rats were used for the study.

Only rats weighing above100g were used.

3.7 Exclusion criteria

Any pregnant or diseased rat as well as those weighing less than 100g were excluded.

3.8 Collection and botanical identification of plant sample

The banana was collected from a single plant and a sample taken to the department of botany he Mbarara University of Science and Technology (MUST) for identification.

3.9 Preparation of green banana peel extract

After harvesting the green banana, it was peeled. The peels were dried on the laboratory bench Pharmacology laboratory for 3days. Partially dried banana peels (1000grams) in 1000 ml of water was boiled for 20 minutes & on cooling it was blended for five minutes, the solution was then filtered in sterile wire guarze to remove the fibers. Then we proceeded to filter through Wattman No.1 filter paper.

The extract was poured in weighed plastic jar; water was evaporated at 100°C to obtain a semisolid crude extract which was then dried in and oven at 60°C to obtain a dry mass.

3.10 Photochemical analysis:

The photochemical analysis of the extract of *banana* was carried out to identify the photochemical constituents present in the extract. Test were carried out using standard methods described by Evans (2002), and Harborne (1998).

TEST	PROCEDURE	OBSERVATION	RESULT/ CONCLUSION
Saponins (frothing test)	To 1ml of the extract were shaken with 5mls of distilled water and then heated to boil.		
Tannins	To 1ml of the extract was mixed with a few drops of ferric chloride		
Reducing sugars (Benedict's test)	To 1ml of extract, 4 drops of Benedict's reagent were added, mixed and heated to boil for 5 minutes.		
Flavonoids	To 1ml of extract, dilute NaOH was added followed by dilute HCl		
Cardiac glycosides	To 1ml of extract, a few drops of glacial acetic acid were added followed by a drop of ferric chloride. 1ml of conc. H_2SO_4 was gently added		

Alkaloids	1ml of extract was acidified with HCl. To the mixture, a few drops of wegners reagent were added		
Ninhydrin test	To 1ml of extract, 3mls of Ninhydrin reagent was added and mixture boiled for a few minutes		
Steroids	To 1ml of extract a few drops of acetic anhydride was added, boiled and cooled. Small amounts of conc. H_2SO_4 was added down side of the tube		
Terpenoids (salkowski test)	1 ml of the extract was mixed with 2mls of chloroform and 3mls of concentrated sulphuric acid. (H_2SO_4) was carefully added to form a layer.		
Phlobatanins.	2mls of the filtrate of the extract were boiled with 2% HCL solution.		
Diterpenes	To 1ml of extract, 3 drops of copper acetate solution were added		
Anthroquinones	To 1ml of extract conc. HCl was added followed by a few drops of 10% FeCl3 and diethyl ether. 1ml of conc. Ammonia solution was added		

Table 1: Experimental description for photochemical analysis

3.11 Determination of Acute toxicity:

- The acute toxicity of the aqueous extract of the green banana was determined by using female albino lab rats; the animals were fasted for 24 hours prior to experiment. Animals were weighed with electronic balance and marked according to different body marks. The animals were grouped into three groups and each group (three animals) will be administered a different dose (Lorke, 1983).
- 200mg/kg, 400mg/kg and 800mg/kg dosages were used in the study.
- Volume to administer was

$$\frac{\text{(Body weight * dose)}}{\text{Concentration * 1000}}$$

- Animals were observed for signs of toxicity after 3 hours and 24 hours
- 9 animals were used.

3.12 Induction of diabetes

a) Rats were weighed, marked and fasted for 24 hours.

b) Diabetes was induced by a single intraperitoneal administration of alloxan monohydarte (140mg/kg) with 4% saline solution (an average of 0.90 mL per test animal). Before administering alloxan, a base line glycemia was recorded from blood taken from puncture of tail vein.

c) After 3days, the animals blood glucose level was determined were tested with strips and glucometer. Rats with blood glucose above 200mg/dl/7.0 mlmols will be considered as diabetic and selected for the proposed study (*Campos et al.*, 2013).

d) One group of rats not administered alloxan and served as normal control.

e) Insulin treatment 0.03IU/kg was used as reference point.

3.13 Anti-Hyperglycemic activity

Albino rats were randomized into 5 groups, each group consisting of six specimens.

- **Group I:** Non-diabetic rats received NS (2ml/kg) served as normal control group.
- **Group II:** Diabetic rats received NS (2ml/kg) served as diabetic control group.
- **Group III:** Diabetic rats received aqueous banana peel extract at a dose of 400mg/kg
- **Group IV:** Diabetic rats received aqueous banana peel extract at a dose of 800mg/kg
- **Group V:** Diabetic rats received insulin at dose of 0.03IU/kg as a control reference.

Administration of normal saline, peel extract and insulin was daily before feeding the animals.

Testing for random blood glucose and weighing of the animals was done every after three days.

The blood sample was collected from the tail and tested with glucometer.

The whole process took carried out in 15 days.

3.14 Data analysis

Data was analyzed by TTest Microsoft Execl 2010. Differences between two means will be detected using the student's *t*-test

3.15 Ethical considerations

The laboratory animals were taken proper care of. An approval was obtained from the School of Pharmacy prior to the research.

3.16 Limitation to the study

Due to limited equipment and resources, the study was limited to anti-hyperglycemic effect of the extract only.

Biochemical, histological and neurological tests were not be involved.

There is limited information about anti hyperglycemic activity of banana peel green extract.

CHAPTER FOUR

4.0 STUDY RESULTS AND FINDINGS

4.1 Percentage yield

Item	Weight/g
Banana peels used	2330
Empty container	85.4
Empty container + contents from extraction before drying	505.6
Empty container + contents after drying	169.1
Extract	= 169.1 - 85.4 = 83.7

Table 2: Percentage yield

Percentage yield =

$$= \frac{\text{Weigh of empty + extract after drying} - \text{weight of empty container}}{\text{Weight of peels used in the extraction}} \times 100$$

$$= \frac{169.1 - 85.4}{2330}$$

$$= 3.6\ \%$$

4.2 phytochemical analysis observation and results

TEST	PROCEDURE	OBSERVATION	RESULT/ CONCLUSION
Saponins (frothing test)	To 1ml of the extract were shaken with 5mls of distilled water and then heated to boil.	Bubbles(frothining) was observed but disappeared on cooling	Sapponins present in small amounts
Tannins	To 1ml of the extract was mixed with a few drops of ferric chloride	Gelatin turned green	Tannins present
Reducing sugars (Benedict's test)	To 1ml of extract, 4 drops of Benedict's reagent were added, mixed and heated to boil for 5 minutes.	Color changed from blue to green and to yellow	Reducing sugars presnt
Flavonoids	To 1ml of extract, dilute NaOH was added followed by dilute HCl	turned yellow on addition of NaOH then colorless on addition of HCl	Flavonoids present
Cardiac glycosides	To 1ml of extract, a few drops of glacial acetic acid were added followed by a drop of ferric chloride. 1ml of conc. H_2SO_4 was gently added	Brown and green rings formed at the interface of two layers test for cardenolids	Cardiac glycosides present
Alkaloids	1ml of extract was acidified with HCl. To the mixture, a few drops of wegners reagent were added	Rose pink color color	Alkaloids present

Ninhydrin test	To 1ml of extract, 3mls of Ninhydrin reagent was added and mixture boiled for a few minutes	Purple color and sweet smell observed	Amino acids present
Steroids	To 1ml of extract a few drops of acetic anhydride was added, boiled and cooled. Small amounts of conc. H_2SO_4 was added down side of the tube	Brown ring formed between two layers	Trace amounts of steroids present
Terpenoids (salkowski test)	1 ml of the extract was mixed with 2mls of chloroform and 3mls of concentrated sulphuric acid. (H_2SO_4) was carefully added to form a layer.	Two slight brown ring layers formed at interface	Trace amounts of Terpenoids present
Phlobatanins.	2mls of the filtrate of the extract were boiled with 2% HCL solution.	No observed change	absent
diterpenes	To 1ml of extract, 3 drops of copper acetate solution were added	Emerald green color observed	Diterpenes presnt
Anthroquinones	To 1ml of extract conc. HCl was added followed by a few drops of 10% FeCl3 and diethyl ether. 1ml of conc. Ammonia solution was added	No change	absent
Phenols	To 1ml of extract 2ml of distilled water was added by few drops of ferric chloride	The red color slightly changed to pale color	Phenols present

Table 3: phytochemical results and observation

4.3 SUMMARY OF PHYTOCHEMICALS

Saponins	+
Tannins	+
Phenols	+
Flavonoids	+
Cardiac glycosides	+
Alkaloids	+
Steroids	Trace
Terpenoids	+
Phlobatanins	–
Anthraquinones	–

Table 4: Summary of phytochemicals

4.4 hypoglycemic activity

Group I

	Day 0		Day 3		Day 6		Day 9		Day 12		Day 15	
	BS	WGT	BS	WGT	BS	WGT	BS	WGT	BS	WGT	BS	WGT
	4.1	134.6	4	134	4	134	3.8	134.3	3.7	134.6	3.7	134.2
	4	131.2	4	131	3.8	131.4	3.6	132	3.8	132.2	3.8	132.3
	3.5	122.5	3.7	122.7	3.8	122.3	3.5	122	3.5	122.5	3.7	122.3
	3.7	128.5	3.6	128.3	3.5	128	3.7	128.1	3.5	128.5	3.8	128.2
	3.3	122.6	3.4	122.3	3.3	122.1	3.6	122.5	3.5	122.7	3.5	122.2
	3.6	125.6	3.6	125.6	3.8	125.3	3.5	125	3.3	124.9	3.7	124.8
	3.7	127.5	3.71	127.1	3.7	127.18	3.6	127.3	3.5	127.5	3.7	127.3
	0.30	4.84	0.24	4.66	0.25	4.86	0.11	5.06	0.17	5.06	0.10	5.11
	0.12	1.98	0.09	1.90	0.10	1.98	0.04	2.06	0.07	2.06	0.02	2.08

'able 5: Non-diabetic rats received saline solution (2ml/kg) served as normal control group

S- blood sugar (mmol/l) **WGT**- weight (g)

I; mean SD; standard deviation SE; standard error

Group II

No	Day 0		Day 3		Day 6		Day 9		Day 12		Day 15	
	Bs	Wgt	Bs	Wgt	Bs	Wgt	Bs	Wgt	Bs	Wgt	Bs	Wgt
1	3.4	135.4	11.9	120.5	-	-	-	-	-	-	-	-
2	3.4	127.7	11.1	116.3	11.2	110	10.8	101	10.9	95.8		-
3	3.8	102.9	12.3	100.3	11.9	99.8	-	-	-	-	-	-
4	3	120.8	13.6	115.8	13.3	111	13.5	101	12.9	98.7	-	-
5	3.9	134.6	10.9	127.5	11.3	120	11	113	11.5	112.3	11	105
6	3.6	133.5	13.3	128.7	13.4	128	13.2	121	12.9	116.3	12.8	109
M	3.51	125.82	12.18	118.18	12.22	113.7	12.1	108.8	12.05	105.78	11.9	106.9
SD	0.35	12.50	1.11	10.31	1.06	10.61	1.42	9.81	1.01	10.04	1.27	2.26
SE	0.13	5.10	0.45	4.210	0.43	4.33	0.58	4.01	0.41	4.10	0.52	0.92

Table 6: Diabetic rats received saline solution (2ml/kg) served as diabetic control group

Group III

No	Day 0		Day 3		Day 6		Day 9		Day 12		Day 15	
	Bs	Wgt	Bs	Wgt	Bs	Wgt	Bs	Wgt	Bs	Wgt	Bs	Wgt
1	3.5	125	13.5	116	13.2	114	-	-	-	-	-	
2	3.4	121	14	113	14	112	13.3	111	-	-	-	-
3	3	130	13.5	116	13.7	114	13.4	113	13	112	13	111
4	3.6	131	13.6	121	13.3	117	13.7	113	12.9	110	-	-
5	4	121	15.3	113	15.5	113	15.6	112	-	-	-	-
6	3.5	138	13.5	127	13	124	13	121	12.8	118	13	116
M	3.5	127.4	13.9	118	13.78	115.7	13.8	114	12.9	113	12.8	113.5
SD	0.322	6.57	0.71	5.18	0.91	4.34	1.03	3.87	0.10	4.12	0.21	3.53
SE	0.132	2.68	0.29	2.12	0.37	1.77	0.42	1.58	0.04	1.68	0.09	1.44

Table 7: Diabetic rats received aqueous banana peel extract at a dose of 400mg/kg

Group IV

Day 0		Day 3		Day 6		Day 9		Day 12		Day 15	
Bs	Wgt	Bs	Wgt	Bs	Wgt	Bs	Wgt	Bs	Wgt	Bs	Wgt
3.7	140.5	16.3	130.2	16.3	124.6	16.5	123.6	15.9	120.1	15.5	115.3
3.3	140.5	15.9	135.1	15.5	130.5	15.4	127.9	15.2	123.2	14.9	118
3.9	130.6	14.8	125.2	14.5	123	14.2	121	14	118.5	14.2	115.3
4	130.1	17.3	125	17	120.8	-	-	-	-	-	-
3.2	128.8	15.4	126	15.3	122.3	15	116.3	15.1	115.1	14.9	113.2
3.5	133.5	16	126.3	16.2	121.7	16	119.5	15.3	117.7	15	115.1
3.6	134	15.95	127.96	15.8	123.81	15.42	121.66	15.1	118.92	14.9	115.38
0.32	5.26	0.84	3.97	0.88	3.51	0.88	4.37	0.68	2.99	0.46	1.71
0.13	2.14	0.34	1.62	0.35	1.43	0.36	1.78	0.28	1.22	0.18	0.69

Table 8: Diabetic rats received aqueous banana peel extract at a dose of 800mg/kg

Group V

Day 0		Day 3		Day 6		Day 9		Day 12		Day 15	
Bs	Wgt	Bs	Wgt	Bs	Wgt	Bs	Wgt	Bs	Wgt	Bs	Wgt
3.7	134.8	17.6	120.5	11	122.6	8.5	126.5	8	128	7.5	129.2
4	114	15.5	100.5	10.5	105.7	8.3	112.7	7.9	113	6	113.5
4	117	16	100.6	10.6	107.8	9.6	113.8	8	114.7	7.2	115.7
3.5	136.5	15.7	116.5	11.1	119.3	8.7	123.6	8.1	127.5	6.9	130.5
3.8	140	15.9	120.5	9.6	123.6	8	127.7	7.5	130	6.8	133.2
3.9	135.5	17.3	120.3	10.9	125.5	7.9	128.5	7.8	129.6	6.5	131.8
3.65	129.63	16.33	113.15	10.61	117.41	8.5	122.13	7.88	123.8	6.81	125.65
0.19	11.13	0.88	9.87	0.54	8.52	0.61	7.08	0.21	7.78	0.52	8.68
0.07	4.54	0.36	4.032	0.22	3.48	0.25	2.89	0.08	3.17	0.21	3.54

Table 9: Diabetic rats received insulin at dose of 0.03IU/kg as a control reference.

BS- blood sugar (mmol/l) **WGT**- weight (g)

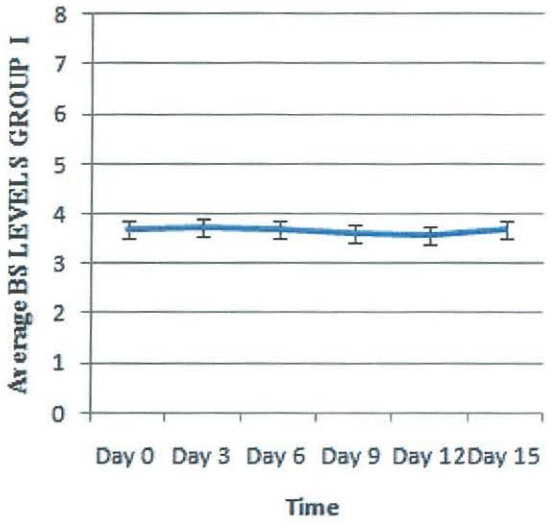

Figure 1: A graph of average BS levels for GROUP I against time

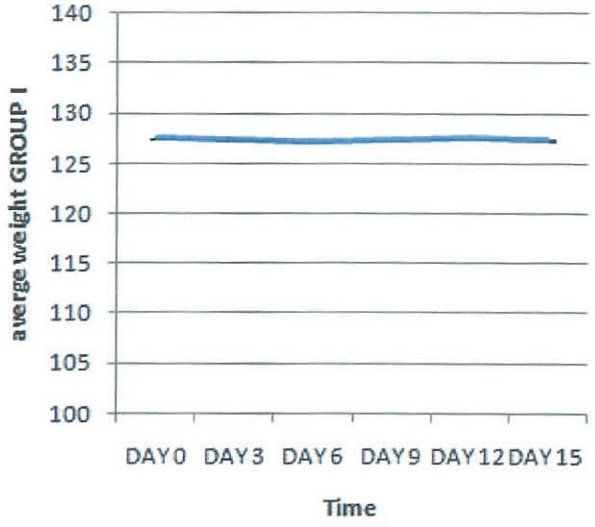

Figure 2: A graph of average wieght for GROUP I against time

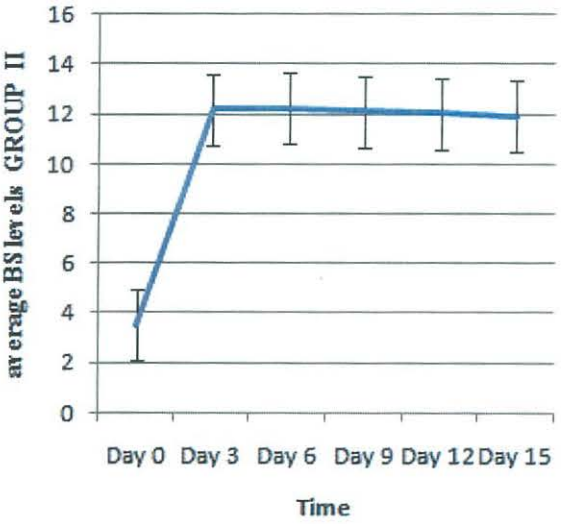

Figure 3: A graph of average BS levels for GROUP II against time

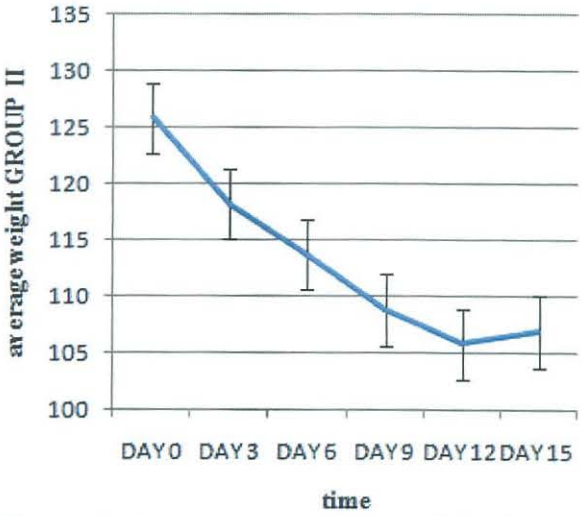

Figure 4: A graph of average weight for GROUP II against time

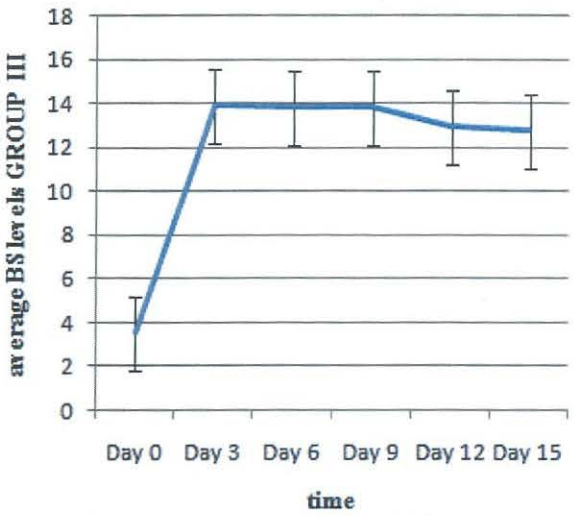

Figure 5: A graph of average BS levels for GROUP III against time

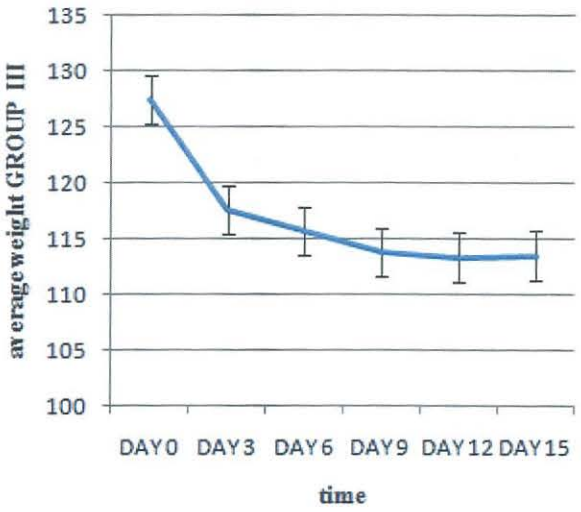

Figure 6: A graph of average weight for GROUP III against time

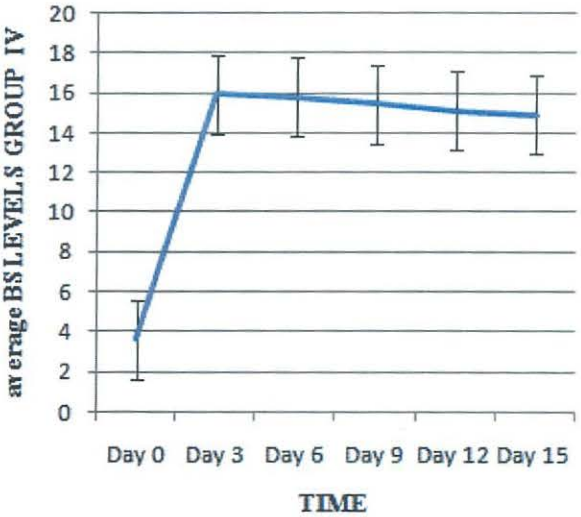

Figure 7: A graph of average BS levels for GROUP IV against time

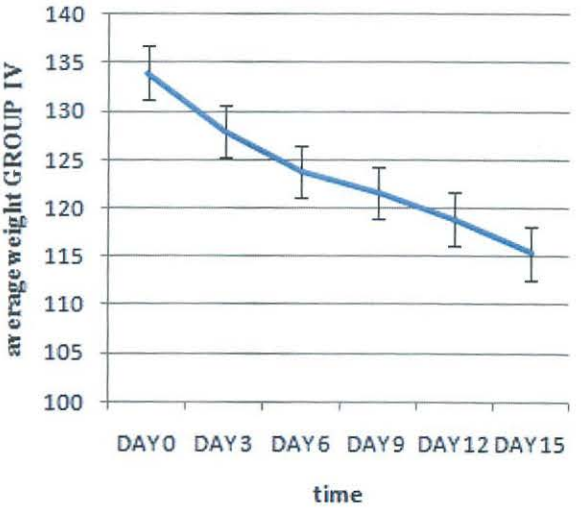

Figure 8: A graph of average weight for GROUP IV against time

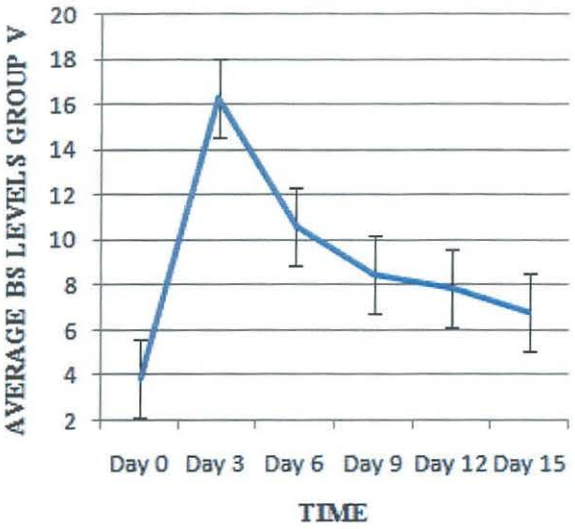

Figure 9: A graph of average BS levels for GROUP V against time

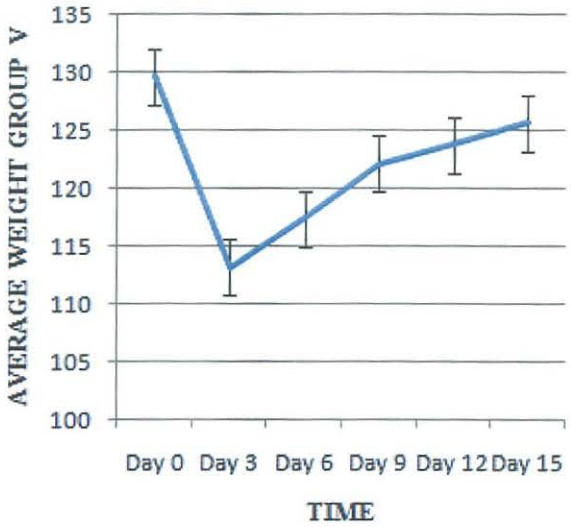

Figure 10: A graph of average weight for GROUP V against time

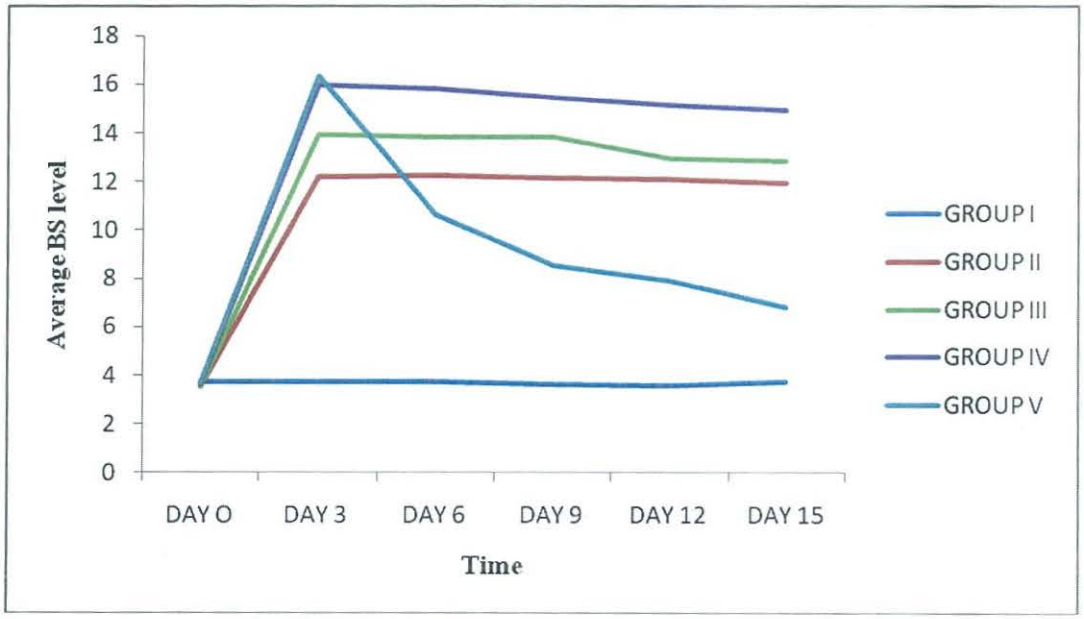

Figure 11: A graph of average BS levels for against time

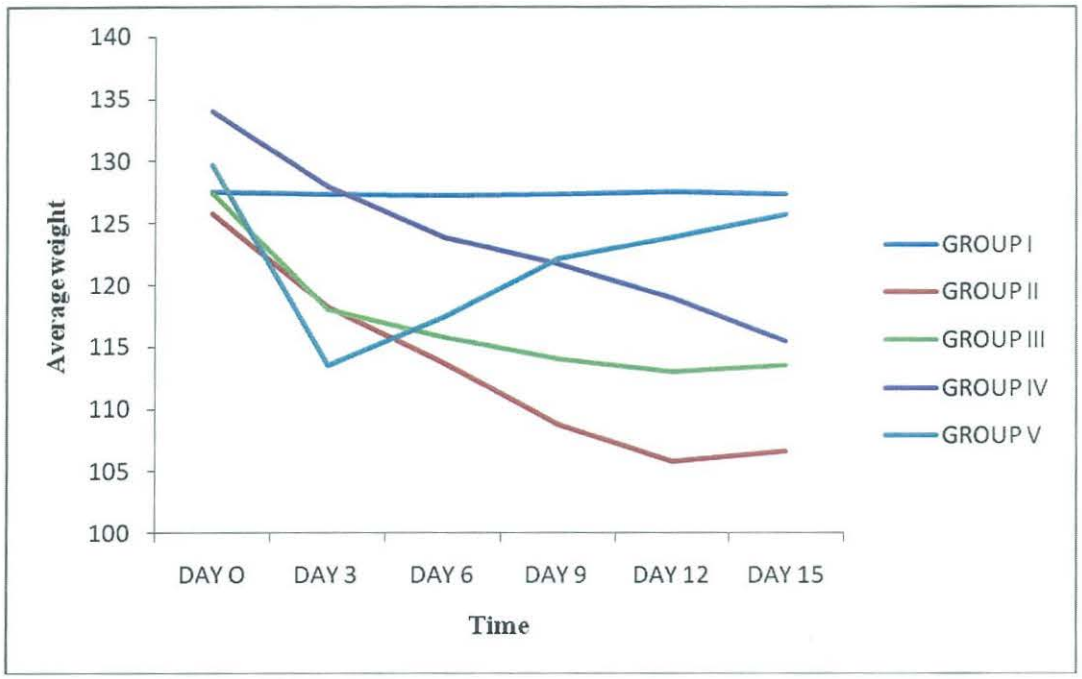

Figure 12: A graph of average weight against time

4.5 DISCUSSION OF RESULTS

Diabetes a condition where there is a manifestation of plasma glucose levels higher than normal. The consensus is clear about that intensive treatment of all factors risk are the best way to prevent or delay diabetes-associated morbidity, that is why reducing hyperglycemia to prevent the occurrence of these effects is the best strategy hypoglycemic activity (Jara., 2006)

Starting from the carbohydrate digestion, breakdown into the monosaccharide, their building blocks glucose, fructose and sucrose to the downhill utilization of glucose for energy. Any disturbance in the normal pathway may lead to impaired glucose metabolism, the onset of hyperglycemia and subsequently DM. the released glucose becomes the primary stimulus of for the beta-cells of the pancreatic islets. However prolonged exposure of glucose to the beta-cells of the pancreatic islets concentration impairs glucose stimulated insulin release. Glucose stimulation of the pancreas initiates a cascade of events resulting into insulin secretion and is dependent on increased intracellular calcium. One of the key effects of insulin is to enhance is to enhance the overall glucose disposal and this is achieved by stimulation of glucose uptake by into target tissue. This is facilitated by insulin sensitive glucose transporter GLUT-4 uniquely expressed in in skeletal muscles, cardiac muscle and adipose tissue. Glucose stimulated release of insulin and insulin guided metabolism of glucose are therefore the primary balancing factor of the glycemic state in the body. However elevated glucose levels in the body results into glucose toxicity this results into production and generation of reactive oxygen species (ROS). Chronic oxidative stress due to hyperglycemia plays an important role in the progressive beta-cell dysfunction. Hyperglycemia alone does not cause diabetic complication, rather due to effect of glucose toxicity due to chronic hyperglycemia and is complicated through oxidative stress. Oxidative stress can produce major interrelated derangements of cell metabolism, including DNA strand breakage, rise in intracellular free Ca2+, damage to membrane ion transport and other specific proteins and peroxidations of lipids. Diabetic hyperglycemia causes pathological changes in small vessels, arteries and peripheral nerves (Asok *et al,* 2008). Oxidative stress has been reported to be the major cause of pathogenesis in various diseases. Oxidative stress causes tissue damage and injury to all molecular targets in DNA, proteins and lipids, thus causing cell death. The oxidative stress and the resultant tissue damage are the hallmark of chronic diseases and cell death and diabetes is no exception. (Banyes and Thorpe., 1999). Complication such as cardiomyopathy, nepthropathy, neuropathy, retinopathy, stroke, diabetic foot, arises.

Currently phytochemicals identified from traditional plants are presenting an exciting opportunity for the development of types of therapeutics. This has led to an increase in the global effort to harness and harvest those medicinal plants that bear substantial amounts of phytochemicals showing multiple beneficial effects in combating diabetes and diabetes related complications. (Siddiqui *et al.,* 2010).

Living organisms have protective system, the so called "antioxidant defensive system" which involves a variety of components, both endogenous and exogenous in origin. These components function interactively and synergistically to neutralize free radicals. It is also reported that dietary intake of antioxidant rich food/herbs decreases the incidence of a number of human disorders. Regular use of banana in the diet provides beneficial effects associated with antioxidants in the body (Pari and Umamaheshwari., 2000)

In living system, varieties of antioxidant mechanisms play an important role in combating ROS. Few of the mechanisms are free radical scavenging, complexation of pro-oxidant metals, reduction and quenching of singlet oxygen formation. Regular use of banana in the diet provides beneficial effects associated with antioxidants in the body (Ashish et al, 2011)

Though different types of oral hypoglycaemic agents are available along with insulin for the treatment of diabetes mellitus, there is an increasing demand of patients to use natural products with antidiabetic activity. Insulin cannot be used orally and continuous use of the synthetic anti-diabetic drugs causes side effects and toxicity (Hye-Jin et al, 2005)

Phytochemical evaluation.

Phytochemicals occur naturally in plants and they are responsible for color and organoleptic properties, protection. Previous reports have indicated that phytoconstituents in fruits and vegetables may reduce the risk of cancer possibly due to dietary fibers, polyphenols, antioxidants and anti-inflammatory effects

This study found after phytochemical screening of the aqueous extract of the banana peel revealed saponins, tannins, phenols, flavonoids, cardiac glycoside, alkaloids, steroids and terpenoids as shown in table 4.

Acute toxicity study; showed no signs of toxicity such as salivation, diarrhea, depression, stimulation, vomiting, and comma and there was no mortality observed up to a dose of 4000mg/kg. So the extract was found to be safe for long term administration.

Normal control group

Plasma levels baseline of glucose are observed in the study groups, ranging from 3.0 to 4.1mmol/L. In normal rats, with free access to food and water, glucose values between 2.5 and 5.5mmol/L (Nitz et al., 2003), although variations may be higher, depending on the strain and the type of food they receive. This shows that our specimens are homogeneous and comparable in status and energy metabolism, and ensures that our results are more reliable

Induction of diabetes, alloxan

Although a pharmacological model cannot be extrapolated to a complex human pathology, the use of alloxan is accepted as a chemical induction model resembles human diabetes (González, 2006). After receiving alloxan to the single intraperitoneal dose of 140mg/kg, the glucose levels in groups II, III, IV and V showed a significant increase ($p < 0.05$) and significantly higher, an increase that exceeds 400% of the values basal glucose. The glucose levels rise to levels ranging from 9.0 to 18.0 mmol/L, however the percentage of diabetic rats obtained is variable and depends on the sensitivity of the rats (Di Loreto, 2003).

Alloxan is the also used chemical for induction of diabetes mellitus. It is a well- known diabetogenic agent widely used to induce Type 1 diabetes in animals. Alloxan is a urea derivative which causes selective necrosis of the pancreatic islet β-cells. This diabetogenic agent forming free radicals, depletion of NAD+ due to DNA damage and inhibition of glucosinase within mitochondrial changes include decreased reduced glutathione, calcium and deficient ATP (Szkudelski, 2001). It is used to produce experimental diabetes in animals such as rabbits, rats and mice. With this agent, it is possible to produce different grades of severity of the disease by varying the dose of alloxan used. Thus alloxan induced diabetes mellitus served as a pathological biomodel for testing a substance with supposed antioxidant activities or anti-diabetic agent.

There was no observed changes in BS levels weight for GROUP I because animals were kept under normal conditions with free access to water to food.

EFFECT OF EXTRACT ON BS LEVELS

Administration of the extract to GROUP III and GROUP IV did not show any significant changes blood sugar levels as compared to that of GROUP V which received the standard drug insulin.

Some phytochemical compounds such as terpenes, tannins, steroids and alkaloids have been implicated in anti-diabetic activities of plants. These constituents may exert activity either singly or in synergy with one another. (shigemori et al, 2003, Ranjitsingh et al,2013). Phytochemical evaluation of the banana peel revealed presence of saponins, tannins, phenols, flavonoids, cardiac glycoside, alkaloids, steroids and terpenoids. Although these constituents in banana peel extract may possess anti-diabetic activity, the whole green fruit may be more effective in DM since the result of bioactivity of did not reveal any increase in magnitude of hypoglycemia.

DIETARY MANAGEMENT OF DM

During diabetes, appropriate nutritional management is essential for restoring and maintaining a good metabolic state. In this context, diet remains a cornerstone in diabetic management. Dietary spices or their active principles have been shown to have a positive influence on the activities against DM. It is known that banana is a good source of bioactive compounds such as flavonoids, such as gallocatechin, with high antioxidant properties (Jyothirmayi et al, 2012) , Manmohan and Purnima. 2012; Joshi 2000; Kanazawa and Sakakibara 2000; Shinichiet al.2002).

Reports indicate that with banana being a tropical plant, it produces large amounts of antioxidants to protect itself from the oxidative stress caused by strong sunshine and high temperature (Shinichiet al.2002). In the banana peel, these antioxidants may be helpful in reducing the complications of diabetes when consumed in the form of diet. Though there are few evidences that state the hypoglycemic effect of the different parts of the banana plant, viz., fruit, root and flower (Gomathyet al.1990; Pari and Umamaheshwari 1998; Joshi 2000; Ojewole and Adewunmi 2003; Matook and Fumo 2005). I undertook the work in this aspect to study the effect of the banana peel (*Musa sp. cultivar 'nakitembe'*) on BS levels in alloxan induced diabetic rats. Although the effect of the extract was not significant on hyperglycemia, the flavonoid content has been demonstrated to possess significant effect on normalization of serum creatinine level and lowering of TG and relative weight of liver, indicating possible presence of kidney and liver protective property (Shukla & Bigoniya, 2014).

Green banana contains high levels of starch, mainly in the form of resistant starch. Raw banana flour presents about 75% of starch, of which 3% consists of rapidly digestible starch, 15% of slowly digestible starch and 57% of resistant starch (Zhang & Hamaker., 2012). By definition, resistant starch (RS) is any starch that is not digested in the small intestine but passes to the large bowel. The digestible starch is susceptible to the action of amylase, while the resistant starch is not (Bezerra *et al.,* 2013). Green bananas offer diabetics a high-energy, low-calorie source of carbohydrates, which meets the glycemic requirements recommended for their diets. They pass through the intestines unchanged which give them the characteristics of an insoluble fiber. High fiber foods and foods that contain resistant starch increase satiety and reduce overall calorie consumption, probably due to their effects on digestion and satiety hormones.

Resistant starch (RS);

- Decreases glycemic and insulinemic responses
- Lowers plasma cholesterol and triglyceride concentrations
- Improves whole body insulin sensitivity
- Increases satiety, and reduces fat storage

The body weight change results depicted in for GROUP II, GROUP III, GROUP IV and GROUP V showed a significant ($p<0.05$) reduction in body weight in alloxan-induced diabetic rats treated with banana peel extract and those rats treated with standard drug-insulin when compared to the untreated diabetic rats. The diabetic rats treated with insulin began to regain weight lost on the six day while the diabetic not treated even those treated with extract kept on losing weight. This might be due to impaired glucose metabolism and excessive breakdown of tissue protein which is an indication of diabetes mellitus. This finding is in agreement with Shalini *et al*., 2009, Semwal *et al*., 2008)

After treatment with insulin, GROUP V showed a significance decrease in BS levels and by the last day of treatment it almost returned to normal. Insulin facilitates entry of glucose into muscle, adipose and several other tissues through facilitated diffusion through a family of receptors, the major transporter used for uptake of glucose (called GLUT-4). Binding of insulin to receptors on such cells leads rapidly to fusion of those vesicles with the plasma membrane and insertion of the glucose transporters, thereby giving the cell an ability to efficiently take up glucose. When blood levels of insulin decrease and insulin receptors are no longer occupied, the glucose transporters are recycled back into the cytoplasm. The actions of insulin (indirect and direct) on cells include: Increased glycogen synthesis form glucose insulin (glycogen a form of

storage of glucose in liver (and muscle) cells), increased lipogenesis, decreased proteolysis, decreased lipolysis, decreased gluconeogenesis (production of glucose from non-sugar substrates), decreased level of autophagy (degradation of damaged organelles, increased amino acid uptake. These clinical actions of insulin are directly useful in reducing high blood glucose levels as in diabetes as well as increase or maintenance of body weight in diabetic animals.

CONCLUSION AND RECOMMENDATIONS

The green banana peel extract hypoglycemic activity is not significant. However it contains phytochemicals which have antioxidant properties useful in preventing complication brought about by glucose toxicity in diabetic patients. The flavonoid content has been demonstrated to possess significant effect on normalization of serum creatinine level and lowering of TG and relative weight of liver, indicating possible presence of kidney and liver protective property. The whole green fruit is more effective in DM due to the resistant starch that's not digested in the small intestine foods but reach the colon, the bacterial flora metabolize these compounds anaerobically in the absence of oxygen. This produces gases (hydrogen, carbon dioxide and methane). The gases are absorbed and excreted by breathing or through the anus.

At the doses investigated, the green banana peel aqueous extract did not possess significant anti-hyperglycemic effect. However, it evidently provided palliative effect on the complications resulting from diabetes mellitus. The green banana peels have an added advantage thus diabetic patients should continue boiling the 'matooke' with the peels.

Further studies should be done to:

- Quantify antioxidants (phytochemicals) present in this cultivar of banana.
- Determine long term effects of aqueous banana extract at higher doses.
- Determine the biochemical, histological and neurological effects of banana peel aqueous extract.

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APPENDICES

WORK PLAN

HAVESTING AND IDENFICATION	SEPTEMBER 2014
EXTRACTION	SEPTEMBER 2014
PHYTO CHEMICAL ANALYSIS	SEPTEMBER 2014
ACUTE TOXICITY STUDIES	SEPTEMBER 2014
ANTIHYPERGLYCEMIC STUDIES	OCTOBER 2014
ANALYSIS OF RESULTS	NOVEMBER- DECEMBER 2014
RESEARCH PRESENTATION	FEB 2015

Table 10: Work plan

BUDGET

ITEM	QTY	COSTS /@	TOTAL COST
Rats	39	10,000	390,000
Diabetes strips	4 boxes	65,000	260,000
Allorzan	1 tin 50g		300,000
Filter paper	I box		30,000
Insulin glargine			50,000
Normal saline	I bottle		2.000
Syringes			50,000
Distilled water	3 liters		10,000
Gloves	I box		15,000
Plant specimen authentification			50,000
Stationary and printing			100,000
Animal feeds			50,000
Specimen			20,000
Library and internet costs			70,000
Cotton			10,000
Transport			100,000
Glucometer			From biochem. Department
Miscellaneous			200,000
TOTAL			1,650,000

Table 11: Budget