ASSESSMENT OF ANTIDIABETIC PROPERTIES OF *MOMORDICA CHARANTIA* POWDER

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DECLARATION

I, Cherylene Wambui, hereby declare that this dissertation is my original work and has not been presented for a degree in any other university.

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DEDICATION

With love, I dedicate this work to my mum Vivian Kabiru, and to my fiancé and son, Peter and Richard Wamiti, who gave me the will and purpose to begin and carry on through this course.

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OPERATIONAL DEFINITIONS

Diabetes mellitus - A group of metabolic diseases in which a person has high blood sugar, either because the body does not produce enough insulin, or because cells do not respond to the insulin that is produced.

Diabetes mellitus Type II - formerly non-insulin-dependent diabetes mellitus (NIDDM) or adult-onset diabetes – is a metabolic disorder characterized by high blood glucose in the context of insulin resistance and relative insulin deficiency.

Non- communicable disease - It is a medical condition or disease which is non-infectious. NCDs are diseases of long duration and generally slow progression that include diabetes. Often referred to as "chronic diseases".

Lifestyle diseases - Diseases that appear to increase in frequency as countries become more industrialized and people live longer including type II Diabetes.

Hyperglycemia- A condition in which an excessive amount of glucose circulates in the blood plasma, and is generally a glucose level higher than10 mmol/l (180 mg/dl).

Diabetogenic drugs- Drugs that produce or cause diabetes.

In-vivo- Experimentation using a whole, living organism.

In-vitro- (test tube experiments) Studies in experimental biology conducted using components of an organism isolated from

their usual biological context permitting a more detailed analysis than can be done with whole organisms.

Blood Glucose- The main sugar found in the blood and the body's main source of energy.

Postprandial- Occurring after a meal.

Islets of Langerhans- Irregular clusters of endocrine cells scattered throughout the tissue of the pancreas that secrete insulin and glucagon.

Beta-cells- A type of cell in the pancreas located in the islets of Langerhans and constitutes the predominant type of cell. They make and release insulin.

LIST OF ABBREVIATIONS

ALT- Alanine transaminase

ANOVA- Analysis of Variance

AST- Aspartate aminotransferase

BG- Bitter Gourd

BM- Bitter Melon

BMR- Basal Metabolic Rate

BW- Body Weight

CVD- Cardiovascular Diseases

DFSNT- Department of Food Science, Nutrition and Technology, University of Nairobi

DNS- Dinitrosalicylic acid

FBG- Fasting Blood Glucose

GDM- Gestation Diabetes Mellitus

HbA1c- Glycated Heamoglobin

HIV- Human Immunodeficiency Virus

IDDM- Insulin Dependent Diabetes Mellitus

IDF- International Diabetes Federation

KDMIC- Kenya Diabetes Management and Information Centre

MC- *Momordica Charantia*

MDGs- Millenium Development Goals

MPHS- Ministry of Public Health and Sanitation, Kenya

NBMC- The National Bitter Melon Council

NCD- Non-communicable disease

NIDDM- Non-insulin Dependent Diabetes Mellitus

OGTT- Oral Glucose Tolerance Test

OHD- Oral Hypoglycemic Drug

PHPT- Department of Public Health, Pharmacology and Toxicology, University of Nairobi

PROSEA- Plant Resources of South-East Asia

STZ- Streptozotocin

UON- University of Nairobi

VLDL- Very Low Density Lipoproteins

WDF- World Diabetes Foundation

WHO- World Health Organization

ABSTRACT

Rapidly increasing diabetes mellitus is becoming a serious threat to mankind health in all parts of the world. With regard to this problem, plants represent a vast source of potentially useful dietary supplements for improving blood glucose control and preventing long-term complications in Type II diabetes mellitus. Plant sources of antidiabetic promoting substances are likely to have no side effects and can counter the high cost and poor availability of the synthetic drugs.

This study was designed to determine the antidiabetic potential *Momordica charantia* as a food supplement using both in vivo and in vitro methods. A survey was conducted to determine the sources, uses and preparation methods of the bitter gourd in the local community. This data was collected with the use of a key informant interview questionnaire. It was established that majority of the fruit's users were of Asian origin. It was also highly recommended for blood sugar control among Type II diabetics.

Methanolic and aqueous extract of the fresh and dried fruit were prepared and their antidiabetic effects studied in vitro, based on their inhibition of the activity of alpha amylase enzyme. Alpha amylase inhibition activity was determined using the 3, 5-dinitrosalicylic acid assay. The amount of maltose released by the enzyme from starch in the presence of the extract was determined and used to calculate the percent inhibition activity. The methanolic extract was found to exhibit the greatest alpha amylase inhibition activity out of the two extracts used (9.71 U/mg).

The blood glucose lowering effect of the dried fruit powder was then determined by administering it orally to normal rats. Glucose tolerance tests were carried out in normal, control and extract treated rats. Two doses of 200 and 400 mg/kg body weight of the powder were administered orally to the study rats. Blood samples were collected from the base of the tails of

each rat and blood sugar levels estimated using a glucose estimation kit. After repeated administration of treatment, the dose of 400 mg/kg was identified as the most effective dose.

These results clearly indicate that dried fruit powder of *Momordica charantia* can be employed at the rate of 400 mg/kg body weight to reduce blood glucose.

CHAPTER ONE: INTRODUCTION

1.1 BACKGROUND INFORMATION

Diabetes is one of the most common non-communicable diseases of the 21st century. In 2007 the global burden of diabetes was estimated to be 246 million people (IDF, 2009). The global burden of diabetes in 2010 was estimated at 285 million and projected to increase to 438 million by the year 2030, if no interventions are put in place (IDF, 2009).

This rise in diabetes is associated with demographic and social changes such as globalization, urbanization, aging population and adoption of unhealthy lifestyles such as consumption of unhealthy diets and physical inactivity. A few decades ago, the industrialised countries were mainly affected, but presently, diabetes mellitus is a problem in developing countries as well (Zimmet, 2001).

The financial burden borne by people with diabetes and their families as a result of this disease depends on their economic status and the social insurance policies of their countries. In the poorest countries, people with diabetes and their families bear almost the whole cost of the medical care (WDF, 2010).

In Kenya, the prevalence of diabetes is estimated to be 3.3% (MPHS, 2010). This figure is based on regional projections and is likely to be an underestimation as over 60% of people diagnosed with diabetes in Kenya usually will have attended the health care facility with seemingly unrelated complaints. Therefore two thirds of people with diabetes do not know they have the disease (IDF, 2007).

There are two main and several minor types of diabetes mellitus. Type I diabetes afflicts 5 % of patients with absolute insulin deficiency, while about 90 % are afflicted by Type II diabetes, which is associated with insulin resistance and obesity (Klomann et al., 2010).

Type II diabetes is a metabolic disorder that results from complex interactions of multiple factors and is characterized by 2 major defects: decreased secretion of insulin by the pancreas and resistance to the action of insulin in various tissues (muscle, liver and adipose), which results in impaired glucose uptake and over time leads to multiple organ damage (Williams & Wilkins, 2007).

Type II diabetes can be controlled by what is termed as "lifestyle factors", like diet and exercise. These changes are most likely to occur with implementation of a coordinated range of interventions to encourage individuals to maintain a healthy weight, participate in daily physical activity, and consume a healthy diet (MPHS, 2010).

Diet is the primary therapy for non-insulin-dependent diabetes mellitus (NIDDM) and is an important adjunctive treatment in insulin-dependent diabetes mellitus (IDDM) (Krawinkel and Keding, 2006). Conventional drugs are often limited to treating just one specific condition, whereas natural remedies - because of the complex array of biochemicals, vitamins and minerals they contain - are remarkably versatile and able to provide relief for a wide range of unrelated conditions (Simple Health Cures, 2011).

The dietary approach reduces the possibility of further development of disease. Fortunately, traditional diets in many parts of the world are well adapted to the current

concepts of NIDDM management: a diet low in fat and high in complex carbohydrates (Gill, 1997).

The population of developing countries often has no access to adequate medical care and drugs due to economic or infrastructure reasons. Therefore, nutrition and dietary measures play a crucial role in the treatment of insulin resistance in these countries (Klomann et al., 2010).

There are a large number of plants and natural biomolecules that have been discussed in literature for their antidiabetic effects. Among the fruits containing high levels of antidiabetic properties is Bitter gourd (*Momordica charantia*). It is traditionally used in the control of diabetes and its complications (Abascal and Yarnell, 2005). It has been demonstrated that bitter gourd juice improves glucose and insulin tolerance (Nerurkar et al., 2008). Animal studies also indicate additional effects of BM in regulating weight gain and lipid metabolism (Yadav et al., 2005; Chen et al., 2003; Senanayake et al., 2004; Chaturvedi et al.,2004; Chan et al., 2005).

Traditional antidiabetic plants might provide new oral hypoglycaemic compounds to counter the high cost of the chemical medicines for many rural populations in developing countries. India is well known for its herbal wealth (Gupta et al., 2011).

As knowledge on bitter gourd increases rapidly, its potential as a component of the diet or a dietary supplement for diabetic and prediabetic patients is increasingly being realised

(Krawinkel et al., 2006). However, no specific suggestions have been made for the consumption of specific foods with hypoglycaemic properties.

Natural α-amylase inhibitors from fruits could offer a good strategy to control the post-prandial hyperglycaemia mainly due to the presence of phenolic compounds and provide an effect without the side effects present in the most available drugs such as abdominal distension, flatulence, meteorism and possibly diarrhoea (Matsui et al., 2001; Kwon et al., 2006; McDougall et al., 2005; Khan et al., 1990; Kotowaroo et al., 2006; Gao et al., 2007; Bhandari et al., 2008).

Development of a low cost hypoglycaemic powder for diabetics will help reduce the high costs of chemical therapies. The present study therefore attempts to assess the possibility of controlling type II Diabetics by regular consumption of a food supplement based on the bitter gourd via in vivo and in vitro methods.

1.2 PROBLEM STATEMENT

Type II Diabetes is the more prevalent form of diabetes in Kenya, and Kenyans are developing it at a younger age than people in developed countries. The age of onset of type II Diabetes in the country is between 45 and 55 years, compared with 64 years in developed countries (MJOTA, 2008).

Epidemiological surveys conducted by the Nairobi-based Diabetic Management and Information Centre (DMIC) (2007) shows an increase in the estimated prevalence of diabetes mellitus in Kenya from 3% in 2003 to above 6% in 2007. These figures could be

higher as diabetes mellitus often goes undiagnosed because many of its symptoms though serious, are often missed or are treated as common ailments (MPHS, 2010).

The complications of undetected and untreated diabetes have huge socio–economic costs (MPHS, 2010). Diabetes is one of the leading causes of blindness, renal failure and lower limb amputation. It also triggers cardiovascular disease which is the leading cause of deaths in diabetes patients.

There is a high proportion of wrongfully diagnosed cases of diabetes in Kenya that end up with irreversible complications that end up imposing huge socio-economic costs- to the individual, family, community and in the health care system- resulting from premature morbidity and mortality. This leads to an underestimate of the disease and therefore little attention is being given to the prevention and control of the disease (MPHS, 2010).

For many patients in Kenya, maintenance of treatment for diabetes is expensive and poses an economic burden to their families. As a result some of these patients do not comply with treatment therefore placing them at a higher risk of developing organ damage. Unfortunately, that require more advanced and more expensive care for diabetes related complications are often the very people who cannot afford such care, taking into consideration that approximately 46% of the Kenyan population lives on less than a dollar a day (MPHS, 2010).

Many chronic diseases including diabetes do not cause sudden death. Rather, they cause progressive illness and debilitation. In this way, they reduce productivity of the individual, draining away their resources. This aggravates poverty (MPHS, 2010).

The costs of treatment and loss of productivity undermine and stunt economic growth and negatively impact on realization of the Millennium Development Goals (MDGs), Vision 2030 and other national development targets (WDF, 2007).

Limited studies on the antidiabetic properties of locally grown varieties have been carried out in the country. The bitterness of the fruit would pose a great challenge in encouraging its regular consumption among diabetic patients hence the need to find ways of reducing this while preserving its antidiabetic activity. It is also not known how storage conditions would affect the mentioned activity of the fruit, a factor important for the transportation and marketing of the powder.

1.3 JUSTIFICATION

Maintaining normal blood glucose in diabetic patients is essential to delay and prevent complications. In comprehensive care of diabetes, blood pressure, lipids, issues pertaining to liver and kidney functions and weight abnormalities should also be managed (MPHS, 2010).

Despite its multi-system effects, diabetes is a controllable disease, and there is unequivocal evidence that its enormous human and economic toll can be significantly reduced by early and aggressive therapeutic intervention (MPHS, 2010).

Up to 80% of Type II diabetes is preventable by changing diet, increasing physical activity and improving the living environment (WDF, 2011). Effective prevention strategies for diabetes are not costly and may actually bring down costs related to other Non-communicable Diseases (NCDs). However, both in health and economic terms, neglecting chronic diseases such as diabetes is very expensive.

Currently, diabetes treatment requires a combination of drugs. These drugs are costly. Furthermore, treatment with drugs is prone to various side-effects such as secondary weight gain, drug–drug interactions and secondary failure. It is therefore beneficial to identify new, less costly and better sustainable therapies that may influence glucose metabolism, have minimal side-effects and similar or more potent efficacies than conventional therapies (Nerurkar, 2008).

Food-based interventions in health have been found to be sustainable especially among the resource poor communities. Although the hypoglycaemic potential of bitter gourd has long been realised, just like other foods with similar properties, no specific recommendations regarding effective levels of consumption have been made.

This study was therefore designed to establish the antidiabetic effects of Bitter Gourd (BG) and evaluate efficacy in reducing glucose levels in rodents as well as its effect against alpha amylase enzyme. This method has been found to be applicable to humans. This was done with the view of establishing the quantities consumable by the individual to be effective in reducing blood glucose levels in humans.

For the present study *Momordica charantia* (MC) was chosen since it is by far the most extensively investigated and most widely acclaimed remedy for treatment of diabetes mellitus since ancient times. In developing countries particularly, where nutrition and dietary measures play a crucial role in the treatment of diabetes mellitus, MC represents a possible means for preventing and treating diabetes mellitus. MC is a cheap fruit that is available the whole year at local markets in tropical Africa (Sridhar et al., 2008; Sekar et al., 2005).

In particular, MC improves glucose tolerance (Leatherdale et al., 1981) and suppresses postprandial hyperglycaemia in rats (Uebanso et al., 2007), and MC extract can enhance insulin sensitivity and lipolysis (Chen at al., 2003; Chen and Li, 2005). Some studies also claimed that the hypoglycaemic effect of MC was comparable with oral medications such as tolbutamide (Sarkar et al., 1996), chlorpropamide (Ojewole et al., 2006) and glibenclamide (Virdi et al., 2003).

Experiments on animals with deleterious substances or in harmful circumstances are very useful and entirely conclusive for the toxicology and hygiene of man. Investigations of medicinal or of toxic substances are also wholly applicable to man from the therapeutic point of view for the effects of these substances are the same on man as on animals, save for differences in degree (La Follett and Shanks, 1995).

There exists a lot of evidence showing the proven effect of compounds found in the bitter gourd in lowering and regulating blood sugar levels. However the bitter components of *M. charantia* characterized as momordicosides and momordicines may make its regular ingestion difficult.

Dried powder of bitter gourd is therefore desirable in practical application as the solid form is more convenient than liquid form; little information exists in literature about the production of dried bitter gourd dried powder (Wu et al., 2010).

Thus, additional evidence is needed to support the efficacy and safety of bitter gourd powder for the management of type II diabetes mellitus (Fuangchan et al., 2011). This study aims to establish the hypoglycaemic effect of locally grown bitter gourd compared with glibenclamide, a chemical drug commonly used for controlling blood sugar in diabetes, and to determine the minimum effective dose of bitter gourd.

1.4 OBJECTIVES

1.4.1 Main Objective

The major objective of this study is to develop an acceptable food powder from *Momordica charantia* and determine its efficacy in controlling blood sugar among Type II diabetics, using rat models.

1.4.2 Specific Objectives

1. To determine the local uses of the bitter gourd through Key Informant Interviews.
2. To determine the enzyme inhibitory activity of methanolic and aqueous extracts of the fruit powder against Alpha-amylase enzyme.
3. To determine changes in fasting blood glucose in the blood samples of the rats under study using an Oral Glucose Tolerance Test (OGTT).
4. To determine the effect of storage time on the antidiabetic property of bitter gourd powder.

1.5 HYPOTHESES

- Aqueous extract of the fruit powder possesses a significant inhibition activity than the methanolic extract on alpha amylase enzyme.

- The dried fruit powder possesses antidiabetic properties comparable to glibenclamide.

CHAPTER TWO: LITERATURE REVIEW

2.1 MOMORDICA CHARANTIA

2.1.1 Description

Vernacular English names of *M. charantia* include bitter gourd, bitter melon, balsam pear, bitter apple, and bitter, African, or wild cucumber. The most popular is "karela," which is used both in India and in East Africa (Keding and Krawinkel, 2006).

It belongs to the family Cucurbitaceae and is a herbaceous, tendril-bearing vine that grows up to 5 meters. It has a distinct warty exterior and an oblong shape. Its seeds and pith appear white in the unripe fruit and are not intensely bitter. As it ripens, its surface colour turns from light green to yellow or orange and the flesh becomes too distasteful to eat (Wikipedia, 2011). Local cultivars are originally from Asia and it is a common cucurbit in the wild flora of Africa (Njoroge and van Luijk, 2004).

2.1.2 Bitter Gourd Species

Momordica comprises about 40 species, the majority of which are African (Njoroge and van Luijk, 2004). Other wild African species include *M. balsamina*, *M. foetida*, and *M. rostrata* (Reyes et al., 1994). The most commonly consumed types are of the China phenotype and the Sub-continent phenotype (Wikipedia, 2010).

2.1.3 Benefits of Bitter Gourd

Nutritional analysis reveals that bitter melon is rich in potassium, calcium, iron, beta-carotene, and vitamins B1, B2, B3 and C (Nutrition and Healing U.K., 2002). It also has a rich amount of Vitamin A, phosphorus, carbohydrates and has good dietary fiber (NBMC, 2008).

Bitter gourd plays a role in the regeneration of and facilitates the recovery of partially destroyed cells; increasing insulin sensitivity of cells; lowering blood sugar and HbA1c levels; and it slows down blood sugar level-dependent cataract formation. Non-diabetic mice showed increased insulin sensitivity and, even under a high-fat diet, no weight gain (Elchebly et al., 1999), a reduced fat cell mass and an increased BMR (Klaman et al., 2000).

Its fruit and seeds are traditionally used as medicinal herbs as anti-HIV, anti-ulcer, anti-inflammatory, anti-leukemic, anti-microbial, anti-diabetic and anti-tumour, to name a few (Taylor, 2002).

2.1.4 Anti-diabetic Components of Bitter Gourd

Momordica charantia fruits contain glycosides, saponins, alkaloids, reducing sugars, resins, phenolic constituents, fixed oil and free acids (Ban Lab Ltd., 2009).

At least three different groups of compounds in bitter melon have been reported to have hypoglycaemic properties, namely a mixture of steroidal saponins known as charantin, insulin-like peptides (Polypeptide-P), and alkaloids. The hypoglycaemic effect is more pronounced in the fruit of bitter melon where these chemicals are found in greater abundance (Rain Tree Nutrition, 2010).

Unripe fruits of bitter melon have been found to have blood sugar lowering capacity, similar to that of insulin and can be used to treat patients with diabetes. The compound that is responsible for this action is charantin.

2.1.4.1 Charantin

A molecule of charantin consists of a glycone or a steroidal portion. It is a whitish crystalline substance, neutral and tasteless and is obtained from the seeds and fruits of bitter melon. It has been proved to offer more positive and successful results than polypeptide-P (insulin-like polypeptide) and tolbutamide (oral hypoglycemic drug) (FutureToday Inc, 2009).

It is highly soluble in relatively non-polar solvent such as chloroform and dichloromethane as well as in apolar solvents like hexane. It is sparingly soluble in water or other highly polar solvents. However, the glucosides attached to its molecules make it slightly soluble in polar organic solvent such as ethanol or methanol (El- Said and Al-Barak, 2011).

Charantin administered at a 50mg/kg dose reduced hyperglycaemia in rabbits by 42% and it was found to possess pancreatic and extra-pancreatic action (PHICL, 2012).

2.1.4.2 Polypeptide-p

Polypeptide-p, also known as P-insulin (or v-insulin, for vegetable insulin), is structurally and pharmacologically comparable to bovine insulin. It is composed of two polypeptide chains bound together by disulphide bonds (PHICL, 2012).

The protein exhibits hypoglycaemic properties and accordingly, compositions comprising the protein can be used for the treatment of hypoglycaemia in mammals (PatentStorm LLC, 2012).

The protein is in the form of an amorphous powder. It is partially water soluble and is unstable as its shelf-life is hardly 2-3 months when kept at normal pressure and temperature (PatentStorm LLC, 2012).

It activates the inactive insulin and, thus, it can rejuvenate the pancreas depending upon the chronicity of the pathological condition of the individual. Numerous experiments have confirmed that the single dose to about 12 mg to 70 mg of the protein at a time is quite effective (PatentStorm LLC, 2012).

2.1.4.3 Alkaloids

Alkaloids are a group of naturally occurring chemical compounds that contain mostly basic nitrogen atoms. Alkaloids are produced by a large variety of organisms, including bacteria, fungi, plants, and animals, and are part of the group of natural products (also called secondary metabolites) (Wikipedia, 2012).

They often have pharmacological effects and are used as medications or as recreational drugs. They almost uniformly invoke a bitter taste (Rhoades, 1979).

Most methods exploit the property of most alkaloids to be soluble in organic solvents but not in water. In the acidic extraction, the raw plant material is processed by a weak acidic solution (e.g., acetic acid in water, ethanol, or methanol) (Wikipedia, 2012).

2.1.4.4 Phenols

Phenolic acids are plant metabolites widely spread throughout the plant kingdom with a potential protective role, through ingestion of fruits and vegetables, against oxidative

damage diseases (coronary heart disease, stroke, and cancers). Plants produced it as a response for defending injured plants against pathogens (Robbins, 2003).

It is thought to have an anti-diabetic effect by means of interactions of phlorotannins with α-amylase and α-glucosidase in the gut. Polyphenols are the most abundant antioxidants in the diet (Scalbert, 2005).

2.2 DIABETES MELLITUS

2.2.1 Description

Diabetes mellitus is a chronic metabolic disorder that occurs when the pancreas does not produce enough insulin, or when the body cannot effectively use the insulin it produces or both. This results in elevated blood sugar (hyperglycaemia) and other metabolic derangements which over time lead to multiple organ damage. The common complications of diabetes include – eye complications, damage to heart, blood vessels, kidneys, nervous system and foot complications leading to amputations (MPHS, 2010).

Type II diabetes is the most common type of diabetes. It is also known as adult-onset diabetes, non-insulin-dependent diabetes mellitus (NIDDM) or maturity-onset diabetes. It accounts for some 85 to 90 percent of all cases of diabetes. Type II diabetes runs in families. One's risk of having type II diabetes goes markedly high if you have excess weight, leads a sedentary lifestyle and have a family history of type II diabetes (Type II Diabetes, 2011).

Insulin resistance, common in people over the age of 40, is the reduced sensitivity of tissues to circulating insulin resulting in reduced glucose consumption by tissues, causing

hyperglycaemia, and eventually leading to metabolic syndrome and diabetes (O'Rourke, 2009; Tanti and Jager, 2009).

2.2.2 Pathology

For most type II diabetics, the pancreas actually produces more insulin than is necessary, at least in the early stages of the illness. The problem is that dietary fat and cholesterol infiltrate the blood and block insulin from making glucose available to cells. As the disorder progresses, the pancreas weakens, and production of insulin diminishes until insulin injections may be necessary. Most type II diabetics do not need insulin, at least in the early or middle stages of the disease (Type II Diabetes, 2011).

Because the glucose is not consumed by cells, blood sugar becomes abnormally high. This results in excessive urination and constant thirst and hunger. The cells are starved of the fuel that is necessary to function. The sufferer will experience excessive fatigue. If allowed to proceed unchecked, this can ultimately lead to the death of cells and the body itself (Type II Diabetes, 2011).

2.2.3 Types of Diabetes

There are three main types of Diabetes:

1. Type I – Previously referred to as Insulin Dependent Diabetes or Autoimmune Diabetes or Juvenile diabetes or early onset diabetes mellitus. It is as a result of failure of the pancreas to produce insulin.

2. Type II – Previously referred to as Non-insulin Dependent or Maturity onset diabetes that results from failure of the pancreas to produce adequate insulin or

failure of body cells to utilize insulin or both. It accounts for about 85- 90% of total diabetes burden.

3. Gestation Diabetes Mellitus (GDM) – Pregnant women who have high blood sugar (glucose) levels during pregnancy are said to have gestational diabetes. Other specific types include diabetes as part of other Endocrine syndromes, drug induced diabetes and pancreatic disease (MPHS, 2010).

2.2.4 Predisposing Factors

Several predisposing factors contribute to the development of type II diabetes including advanced age, a family history, excessive body weight and alcohol consumption, physical inactivity, stress and consumption of an unhealthy diet (MPHS, 2010).

Several modifiable risk factors associated with urbanization come to fore as driving forces of the rising prevalence of type II diabetes in Kenya. These common urban events and lifestyles are now reaching rural Kenya (MPHS, 2010).

These factors include: consumption of refined carbohydrate; consumption of high-fat diets; lack of physical activity due to sedentary lifestyles, lack of exercise or circumstantial reduction of physical exercises occasioned by the availability of motorized transport, watching television and computer games for long hours (MPHs, 2010).

2.2.5 Prevalence of Diabetes in Kenya

Non-communicable diseases such as diabetes, cardiovascular diseases and cancers, and their related risk factors such as high blood pressure, high cholesterol, and excessive bodyweight are increasing in Kenya (MPHS, 2010).

Like other developing countries, Kenya is experiencing this emerging diabetes epidemic. It is estimated that the prevalence of diabetes in the country is about 3.3%. This figure is projected to rise to 4.5% by 2025 if this trend is not checked (MPHS, 2010).

In Kenya, the estimated figure of the prevalence of diabetes is based on regional projections and is likely to be an underestimation as over 60% of people diagnosed to have diabetes in Kenya usually present to the health care facility with seemingly unrelated complaints. Therefore two thirds of people with diabetes do not know they have the disease (IDF, 2007).

According to Ministry of Public Health and Sanitation (2010), the types of diabetes in Kenya constitute the following proportions:

1. Type I- This type comprises about 10-15% of total diabetes burden.
2. Type II- It accounts for about 85- 90% of total diabetes burden.
3. Gestation Diabetes Mellitus (GDM) - affects about 4% of all pregnant women.

2.2.6 Control Strategies

Treatment: The three methods of treatment are diet alone; diet and oral hypoglycaemic drugs; diet and insulin. Approximately 40% of new cases of diabetes can be controlled adequately by diet alone, about 30% require insulin and another 30% need an oral hypoglycaemic drug.

Management: Other approaches to diabetes management include physical exercise, psychological support, and the monitoring of blood glucose (MPHS, 2010).

2.2.6.1 Oral hypoglycaemic drugs

There are different classes of anti-diabetic drugs, and their selection depends on the nature of the diabetes, age and situation of the person, as well as other factors (Wikipedia, 2011).

Treatments include (1) agents which increase the amount of insulin secreted by the pancreas, (2) agents which increase the sensitivity of target organs to insulin and (3) agents which decrease the rate at which glucose is absorbed from the gastrointestinal tract (Dungan, 2010).

a) Sensitizers

Insulin sensitizers address the core problem in type II diabetes- insulin resistance.

Biguanides: These reduce hepatic glucose output and increase uptake of glucose by the periphery, including skeletal muscle. Metformin, a biguanide, has become the most commonly used agent for type II diabetes in children and teenagers and is the only widely used oral drug that does not cause weight gain. Typical reductions in HbA1c values for metformin are 1.5-2.0% (Higgins, 2010).

Thiazolidinediones: Thiazolidinediones such as Avandia (Rosiglitazone) reverse insulin resistance by acting on muscle, fat and to a lesser extent liver to increase glucose utilization and diminish glucose production (Higgins, 2010).

b) Secretagogues

Insulin secretagogues trigger insulin release by inhibiting the KATP channel of the pancreatic beta cells.

Sulfonylureas: These work by stimulating endogenous release of insulin. They work best for patients over 40 years old having Diabetes Mellitus for under ten years.

Typical reductions in HbA1c values for second generation sulfonylureas are 1.0-2.0%.

First-generation agents include: tolbutamide, acetohexamide, tolazamide and chlorpropamide .

Examples of second-generation agents include: glipizide, glyburide (glibenclamide), glimepiride and gliclazide (Higgins, 2010).

c) Alpha-glucosidase inhibitors

They inhibit the enzyme alpha glucosidase, which helps to absorb glucose into blood stream in the level of intestine cells. Thus, it is possible to slow or inhibit glucose absorption, and reduced blood sugar levels after meals (All about beating diabetes.com, 2010).

These agents are effective by themselves only in the earliest stages of impaired glucose tolerance, but can be helpful in combination with other agents in Type II diabetes (Wikipedia, 2011).

Typical reductions in HbA1c values are 0.5-1.0%. Examples of these agents include: miglitl (Glyset) and acarbose (Precose/Glucobay). They do have the potential to cause weight loss by lowering the amount of sugar metabolized (Wikipedia, 2011).

Disadvantages/side effects

The possible side effects that may be experienced with the use of these drugs include: risk of lactic acidosis, a metallic taste in the mouth, mild anorexia, nausea, abdominal discomfort, diarrhoea; an increase in the number of severe cardiac events (rosiglitazone); skin reactions, and abnormal liver function and weight gain (sulfonylureas) (Higgins, 2010).

An unpleasant flushing reaction after alcohol ingestion and hyponatremia (low blood sodium) can occur with the use of chlorpropamide. The main side effects of alpha-glucosidase inhibitors are flatulence and diarrhoea (Wikipedia, 2011).

2.2.7 Most studied antidiabetic medicinal plants

Many plants have been tested and their blood glucose lowering effects confirmed. The most common include: Allium cepa (Onion), Allium sativum (Garlic), Aloe vera, Caesalpinia bonducella (fever nut), Cinnamomum tamala (Bay leaf,), Coccinia indica (Ivy gourd), Gymnema sylvestre (Gurmar), *Momordica charantia* (Bitter Melon), Murraya koeingii (curry tree), Ocimum sanctum (Holy basil), Panax (Asian) Ginseng, Trigonella foenum-graecum (Fenugreek), Pterocarpus marsupium (Indian Kino) and Syzigium cumini (Jamun) (Bailey and Day, 1989; Grover et al., 2002; Bnouham et al., 2006).

2.3 USE OF ANIMALS IN DIABETES STUDIES

Some diseases and health problems involve processes that can only be studied in a living organism. Animals are necessary in medical research when it is impractical or unethical to use humans (APS, 2006).

Animals make good research subjects for a variety of reasons. Animals are biologically similar to humans, they are susceptible to many of the same health problems, and they have short life-cycles so they can easily be studied throughout their whole life-span or across several generations (APS, 2006).

In addition, scientists can easily control the environment around the animal (diet, temperature, lighting, etc.), which would be difficult to do with people. However, the most important reason why animals are used is that it would be wrong to deliberately expose human beings to health risks in order to observe the course of a disease (APS, 2006).

Type II diabetes can be induced in animals by the administration of diabetogenic drugs such as alloxan, streptozotocin, ditizona and anti-insulin serum, with Alloxan being one of the most commonly used models of experimental diabetes (Ban Lab Ltd., 2009).

The blood glucose of normal, healthy rats varies between 50 and 135 mg/dL depending on the type of food consumed and time since the last meal. A blood glucose level of above 135 mg/dL in fasted rats may be indicative of the presence of diabetes.

A blood sample is obtained from a saphenous (back leg) vein, the tail vein or a toenail. A glucometer is used to measure the blood glucose. Fasting blood glucose for rats ranges from 50 to 109 mg/dL.

2.3.1 Animal Models

Animal models have greatly contributed to the study of diabetes mellitus. They give researchers the opportunity to control in vivo the genetic and environmental factors that may influence the development of the disease and thus gain new information on its treatment in humans (Javia, 2012).

Animal models develop diabetes either spontaneously or by using chemical, surgical, genetic or other techniques, and depict many clinical features or related phenotypes of the disease (Javia, 2012).

Diabetes research in humans is impeded by obvious ethical considerations, because provocation of disease is strictly impermissible in man. Animal models of diabetes are therefore greatly useful and advantageous in biomedical studies because they offer promise of new insights into human diabetes (Javia, 2012).

There are a number of animal models used; those that are commonly used will be highlighted.

2.3.1.1 Oral glucose loading animal model

This is referred to as physiological induction of diabetes mellitus because the blood glucose level of the animal is transiently increased with no damage to the pancreas. In the

clinical setting, it is known as Glucose tolerance testing (GTT): a standard procedure often used for the diagnosis of border line diabetes (Etuk, 2010).

In this procedure, study animals are usually divided into experimental and control groups. The animals are then fasted overnight (Choi, 2004). Experimental animals are given a plant extract under investigation while control animals are given a vehicle.

An hour later, both groups of animals are given a fixed amount of glucose, sucrose or starch. Thereafter, blood glucose levels are measured at 0.5, 1, 2 and 3 hours after administration of the carbohydrate. The areas below the oral glucose tolerance curves of experimental and control groups are calculated and compared to determine whether or not the plant extract contribute to the delay in carbohydrate digestion and subsequent lowering of the blood glucose level glucose (Dimo et al., 2007; Hannan et al., 2007).

2.3.1.2 Normoglycemic animal model

Normal healthy animals are used to test potential of oral hypoglycaemic agents, and these are often used in addition to diabetic animal models (Williamson et al., 1996).

The test animals have an intact pancreatic activity and have free access to normal diet till the experiment. The test drug is then administered and blood withdrawn at 1,2,3,4,5,48 and 72 hours after treatment. Blood glucose level is determined.

2.3.1.3 Chemically induced diabetes

A chemical which induces diabetes is called a diabetogenic agent and these are classified into three categories: agents that specifically damage β- cell, cause temporary inhibition of insulin production and/ or secretion and diminish the metabolic efficacy of insulin in

target tissue (Javia, 2012). Among these, alloxan monohydrate and streptozotocin (STZ) are most commonly used.

2.4 CASE STUDIES

Klomann et al. (2010) found that in comparison with the aqueous and the lipid fractions given at 150 mg/kg, the saponin fraction was the most effective water-soluble compounds of BG when treating type II diabetic mice.

Research done by Virdi et al (2003) showed that in comparison with Methanol and Chloroform extracts of the dried fruit, a water extract from fresh fruit given at doses of 20 mg/kg of body weight after freeze drying, gave the best results, resonating with the findings from a similar study by Klomann et al. (2010), where diabetic mice treated with the hydrophilic residue also had lower levels of glycated haemoglobin in comparison with the control mice.

Further glucose lowering effects were found with ethanolic plant extracts, fresh fruit extracts, and acetone extracts of whole fruit powder (Singh et al., 1989).

Research done by Njoroge and van Luijk (2004) had contradictory findings regarding the mechanism of the bitter gourd's activity where significant studies established its effect as being more acute and transient than cumulative and in another, a cumulative and gradual hypoglycaemic effect was found in diabetic patients using the aqueous extract at the end of a 3week trial. The authors also found contradicting findings in a study in which bitter gourd, in the form of fresh juice, dried powder or the powder given as a tablet, did not have any beneficial influence on diabetic patients (Njoroge and van Luijk, 2004).

Today, processed bitter gourd in the form of capsules or tablets is commonly advertised and sold. However, a warning had been released with regard to the use of bitter gourd capsules, because it is not yet known as its advertisers have not indicated the safe dosage when taken with other antidiabetic agents (Diabetes UK Statement on Karela Capsules, 2006).

Data obtained from in vitro and in vivo studies show positive effects of BG on insulin sensitivity (Yibchok-anun et al., 2006). However, some results are contradictory, and the knowledge about active substances, the most effective dosage and the biochemical mechanism is still insufficient (Klomann et al., 2010).

A study by Jayasooriya et al. (2000) found a significant reduction in the concentration of serum glucose in rats fed with diets supplemented with bitter melon powder at 0.5% as compared with those with no added bitter melon, although increasing amounts of this dietary ingredient did not cause any additional hypoglycaemic effects.

To develop an effective and safe application, it is necessary to study the effects of the aqueous and methanolic extracts of the dried powder in a dose dependent manner in order to identify one that would effectively exhibit these properties in Type II diabetes.

CHAPTER THREE: STUDY DESIGN AND METHODOLOGY

3.1 STUDY DESIGN

The study employed an approach as shown in figure 2 that involved: extraction of the dried product; determining the anti-diabetic activity of the extracts; determining the shelf life of the product; feeding of the powder to the rats and determining the changes in blood glucose levels of lab rats upon consumption of dried product.

3.2 MATERIALS

3.2.1 Materials

Green, unripe fresh fruits of the Sub-continent phenotype Bitter gourd were purchased in sufficient quantity from Ngara Market, Parklands area and then transported to University of Nairobi Department of Food Science, Nutrition and Technology for analysis. The variety used was identified as *Momordica charantia linn* (hybrid variety).

3.3 METHODOLOGY

3.3.1 Sampling for Field Survey

Purposive sampling was done to determine the area to be studied, in this case, Ngara Market in Nairobi. It was purposively selected as the bitter gourd is easily found and sold there in sufficient quantities. Ngara was also chosen due to its proximity to the Parklands residential area, inhabited mainly by members of the Asian community, who make up the greatest percentage of bitter gourd consumers in Kenya.

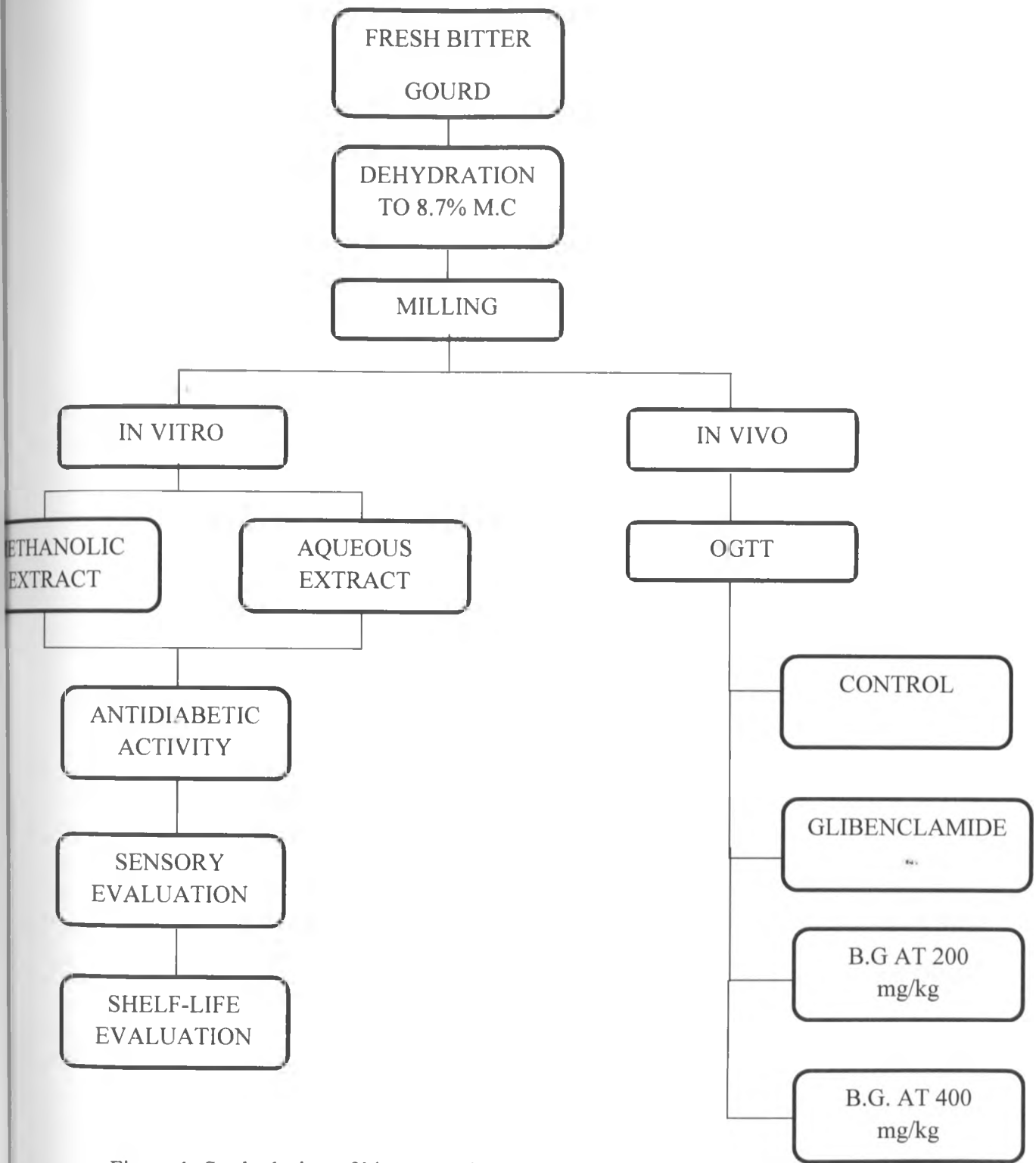

Figure 1: Study design of bitter gourd production, analysis and testing.

Source: Author's conceptualisation.

A key informant question guide was prepared and used to capture information on how the Asian community uses the fruit, the preparation methods and how they relate it to the management of Diabetes Mellitus. It was also used to determine other locally available foods that are used to treat Diabetes. Three respondents from the Asian community and three others who were vendors of the fruit were purposely chosen to be interviewed

3.3.2 Production of the Fruit Powder

The fruit samples were rinsed in tap water to remove dust and foreign material. These were cut into ring-shaped pieces, each measuring approximately half a centimetre in thickness and 3.5 cm in diameter. These were then blanched by immersing in boiling water (B.P. of 94°C ± 1° C) for exactly 4 minutes. An air oven was used to dry the samples at a temperature of 75°C, overnight. The dried samples were then ground using a coffee grinder and sieved in a 600 micron sieve to get a fine powder for further analyses. A sub-sample was weighed, dried at 50°C and reweighed to obtain the dry weight/wet weight ratio.

3.3.3 Sensory Evaluation of the Fruit Powder

A sensory evaluation questionnaire was used to collect information on the organoleptic properties and hence the acceptability of the powder for use as an antidiabetic agent when eaten with meals. The sensory attributes scored were namely Taste, Aroma and Overall Acceptability.

Samples of the product were stored at 25 °C. The powder was then weighed into 3 different amounts (5, 10 and 15 gms). These samples were mixed with a meal of maize and beans (githeri) placed into three bowls having a capacity of 270 grams.

Sensory responses were used to determine the effect of drying on the organoleptic properties of the product as well as the effect of the storage temperature. A seven point Hedonic Scale was used to assess taste, aroma and overall acceptability of the product by 5 panelists.

The values of the Scale used are as follows:

1= dislike very much
2= dislike moderately
3= dislike slightly
4= neither like nor dislike
5= like slightly
6= like moderately
7= like very much

3.3.4 Accelerated Shelf Life Study

Eighteen samples were stored at 55 °C, whereby three samples would be analysed per day for anti-diabetic activity using the α-amylase enzyme assay. The analysis was carried out after every 2 days for a total of 6 days where each day at 55 °C would represent a month of storage at 25 °C.

3.3.5 Rat Study

A total of 18 Wistar rats of either sex weighing 150–180 g were bred in the UON's Kabete campus' Public Health Pharmacology and Toxicology (PHPT) department to attain the age of 6 weeks. The rats were housed in air-conditioned animal house and fed a standard pellet diet and boiled water ad libitum.

3.3.5.1 Oral glucose tolerance testing

Twelve rats were assigned into 4 groups of 3 rats each: the control group was given water; the 2nd Glibenclamide, a synthetic drug at 5 mg/kg; the 3rd and 4th were administered fruit powder in distilled water at dosages of 200mg/kg and 400mg/kg respectively. The rats of all the groups were orally loaded with 2 g/kg glucose 15 min after extract and drug administration.

3.3.5.2 Clinical Examination of Experimental Rats

Blood was collected from the base of the alloxan treated rats' tails by pricking to determine baseline blood glucose levels using a glucometer (Soft Style). Rats showing blood glucose levels above 11.1 mmol/L after 36 hours after a 16 hour fast (Abdel-Rahman, 2011) would be considered diabetic and selected for the study.

Blood samples were collected from the tail prior to drug administration and at 0, 30, 60 and 120 min after glucose loading. Blood glucose levels were measured using Soft Style Glucometer.

3.3.6 Analytical Methods

3.3.6.1 Extract preparation

Methanolic Extract

Methanol was used for one extract keeping in mind that certain active principles like charantin, a phytosterol, can be extracted with methanol (Virdi et al., 2003).

This was prepared using a modified method by Virdi et al. (2003) whereby 0.1 kg of dried fruit of bitter gourd was extracted using methanol at a ratio of 1:10, at 50 °C for 1 h

with stirring at regular intervals. This was then filtered using a Whatman 41 filter paper, the filtrate weighed and evaporated to near dryness. This method was carried out in triplicate, and the average of the residue recorded. The extracts were kept in air tight containers and frozen at -44°C till further analysis was to be carried out.

Aqueous Extract

This was also prepared using a modified method by Virdi et al. (2003), prepared by extracting 0.1 kg of fresh green fruit of bitter gourd along with its seeds using water at a ratio of 10:25. Pieces of fruits were soaked in water for 1 h at room temperature. This was filtered using a course sieve and evaporated to near dryness. This was carried out in triplicate. The extracts were kept in air tight containers and frozen at -44°C till further analysis was to be carried out.

3.3.6.2 In vitro determination of antidiabetic activity

The α-amylase inhibitory activity was determined by an assay modified from the Worthington Enzyme Manual (1993). The number of micromoles of maltose released was determined from a maltose standard curve (appendix 3).

A total of 500 µl of aqueous bitter gourd extract and 500 µl of 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) containing α-amylase (Sigma-Aldrich) solution (0.5 mg/mL) were incubated in test-tubes at 25 °C for 20 min. After pre-incubation, 500 µl of a 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) was added to each tube at timed intervals. The reaction was stopped with 1.0 mL of dinitrosalicylic acid colour reagent (Sigma-Aldrich). The test tubes were then incubated in boiling water bath for exactly 5 min and cooled to room temperature. The reaction

mixture was then diluted with 10 mL of distilled water, and the absorbance was measured at 540 nm using a spectrophotometer (C E 4400 U.V. Vis Double Beam Scanning Spectrophotometer).

This method was applied for the methanolic extract as well. Each experiment was performed in triplicate for both extracts and the average readings taken to determine the amount of micromoles of maltose released. The α-amylase inhibitory activity (Unit/mg) was then calculated as follows:

$$\text{Inhibition Activity} = \frac{C \times d.f.}{V \times T}$$

where C = concentration of maltose released

d.f. = dilution factor

V = volume of enzyme used

T = time in minutes.

3.3.7 Data Analysis

All analyses were performed in triplicate (n = 3), and the data were presented as means standard error of means (± SEM). The results obtained were analyzed by using Statistical Package for Social Sciences (SPSS) where a one sample t-test, a one-way analysis of variance (ANOVA) and Analysis of Covariance tests were used to conduct statistical analyses. Differences were considered significant at $p < 0.05$.

CHAPTER FOUR: RESULTS

4.1 LOCAL USES OF THE BITTER GOURD

Information gathered from responses of market vendors at the Ngara Market in Nairobi revealed that the fruit was identified with the adopted name Karela and it is sourced from Matuu town, Yatta Constituency, in the Eastern Province of Kenya, where it is grown, and thereafter transported to Wakulima Market in the Central Business District of Nairobi. The vendors then source the fruits from the said market for sale.

On inquiry over its preparation, the vendors revealed that the fruit is first washed, cut into cubes with its peel and the seeds removed. This can then be boiled and the solution drank; it can be blended to make a juice or it can be cooked in a similar manner to other vegetables (stewed or shallow fried).

All the interviewees (100%) believed that the bitter gourd was primarily used to control or bring down the levels of blood sugar in diabetic persons. The most appropriate method of its intake as a remedy for the said condition was a glass of the fruit's juice taken in the morning and another in the evening, after meals. The juice is taken when freshly made in order to preserve its potency.

The survey revealed a collective response on the fact that the bitter gourd is fairly effective in controlling blood sugar on its own, albeit it should be used together with a supporting diet and conventional diabetes medicine. This is because on its own as a larger dose, it may lower blood sugars down to dangerous levels and would require an agent to stabilize the blood sugar levels.

Other food based approaches employed in the management of type II Diabetes were listed as French beans, broccoli and celery. However, when compared to the bitter gourd, the latter was found to be most effective. Dietary practices stated as best in controlling the condition included cooking foods with minimal amounts of oil, small food portions eaten 5 times in a day, a diet with large vegetable portions, and smaller ones of protein and carbohydrate foods.

Interviews with selected members of the Asian community revealed that it is mainly used in making curries, crisps and juice. Those who juiced it reported taking it on a daily basis while curries were prepared once or twice in a month.

The curries were prepared by peeling the skin, marinating in salt for half an hour then washing in order to remove the bitterness. These were then cut into slices and fried with onions. Once cooked, some jaggery is added to improve on its taste. As for preparation of the crisps, slices of the fruit are sun dried and stored in airtight containers to be fried when needed.

Persons who used it as a remedy for diabetes attributed its success as a hypoglycaemic agent to the fact that it had insulin like compounds and had no side effects.

4.2 IN VITRO ANTIDIABETIC ACTIVITY DETERMINATION

4.2.1 Moisture Determination of *Momordica charantia*

Table 1 below shows the results of the moisture content determined for fresh, blanched and dried samples.

Table 1: Moisture content of fresh, blanched and dried matter

Wet moisture content (%)	Moisture content of blanched material (%)	Dry matter moisture content (%)
90.5± 1.55	92.37± 0.19	8.703± 0.081

Values are mean and ± standard error of deviation of 3 separate determinations (n=3).

4.2.2 Yield of extracts on Extraction

The yield of the extracted powder was calculated on the basis of dry matter and the results are shown in Table 2 below.

Table 2: Percentage yield of *M. charantia* extracts

Extractant	Mass Extracted (g)	Amount of Dilutant (ml)	% Yield
Methanol	100	1000	15.47± 0.81
Aqueous	100	250	0.86± 0.31

Values are mean and ± standard error of deviation of 3 separate determinations (n=3).

4.2.3 α-Amylase Inhibition Activity

In this study, aqueous and methanolic extracts of dried fruit of *Momordica charantia* were evaluated in relation to the possible inhibition of this enzyme using in vitro assays. The amount of maltose released was determined with the help of a maltose standard curve (appendix 3). The amount determined was then used to calculate the inhibition activity.

An inhibition activity 9.71 Unit/mg was observed for the methanolic extract while the aqueous extract exhibited an activity of 0.08 Unit/mg. The dose dependent responses of α- amylase inhibitory activity of the aqueous and methanolic extracts is shown in figures 3 and 4 respectively.

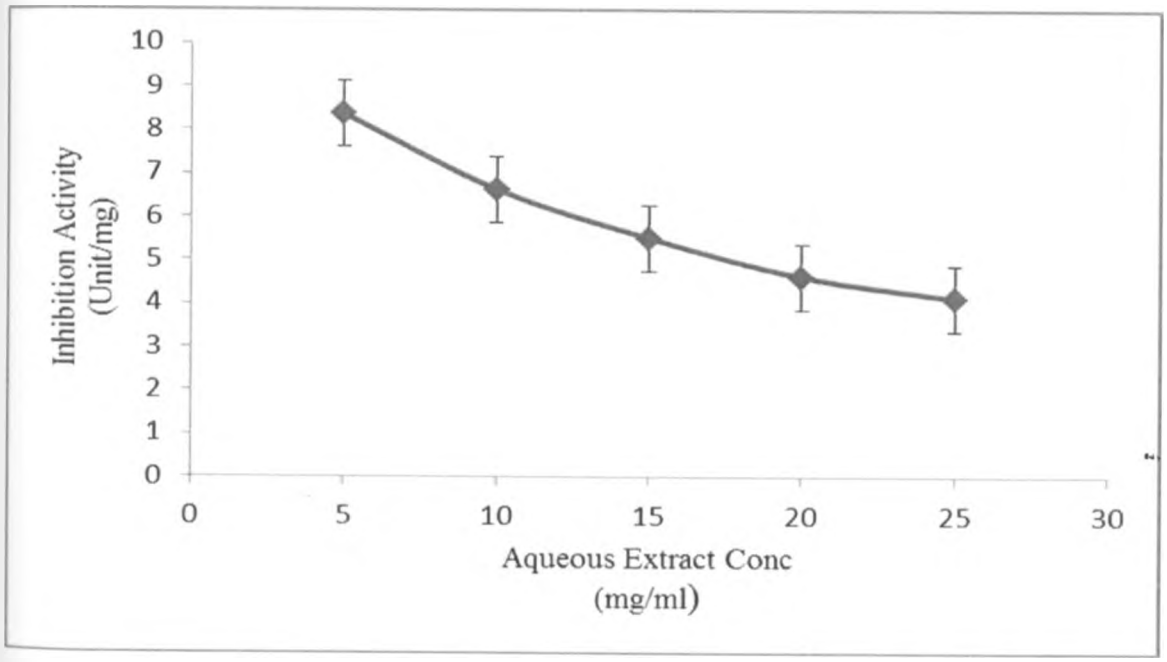

Figure 2: Dose dependent changes in alpha amylase inhibitory activity of aqueous extract. (n=3).

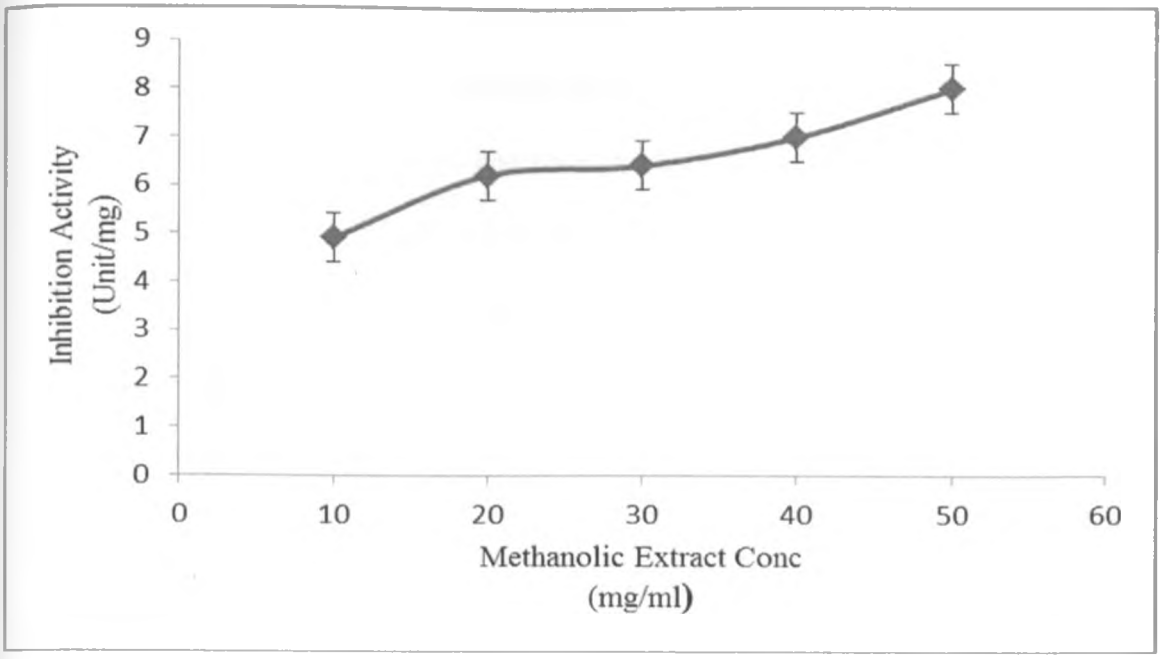

Figure 3: Dose dependent changes in alpha amylase inhibitory activity of methanolic extract. (n=3)

4.3 Sensory Evaluation of Dried Powder

Four attributes (colour, taste, mouthfeel and overall acceptability) were used to determine the dried powder's sensory profile. The results are shown in Table 3.

Table 3: Scores of sensory attributes of M. charantia powder

Level of B.G. (g)	Sensory Parameters		
	Taste	Aroma	Overall Acceptability
5	6.5^{a}	6.2^{a}	6.5^{a}
10	6.7^{b}	6.3^{a}	6.5^{a}
15	6.8^{b}	6.6^{b}	6.7^{a}

Mean values with different superscripts in the same column are significantly different ($p<0.05$)

There was a significant difference in taste and aroma between the three levels of bitter gourd (0.35 and 0.47 resp.) and no significant difference between the levels for overall acceptability (0.63) where $p<0.05$. However, when carrying out homogenous tests for the mean scores, there were similarities in taste for the levels of 10 and 15 gms; there were similarities in the levels of 5 and 10 gms in terms of aroma. The scores for overall acceptability were all homogeneous. This indicated by the superscripts in Table 3.

4.4 ACCELERATED SHELF-LIFE EVALUATION OF DRIED POWDER

4.4.1 Changes in the Antidiabetic Activity of Stored Samples

One day of storage at 55° C represents 1 month in a year. Figure 7 shows the changes in the powder's antidiabetic activity during storage.

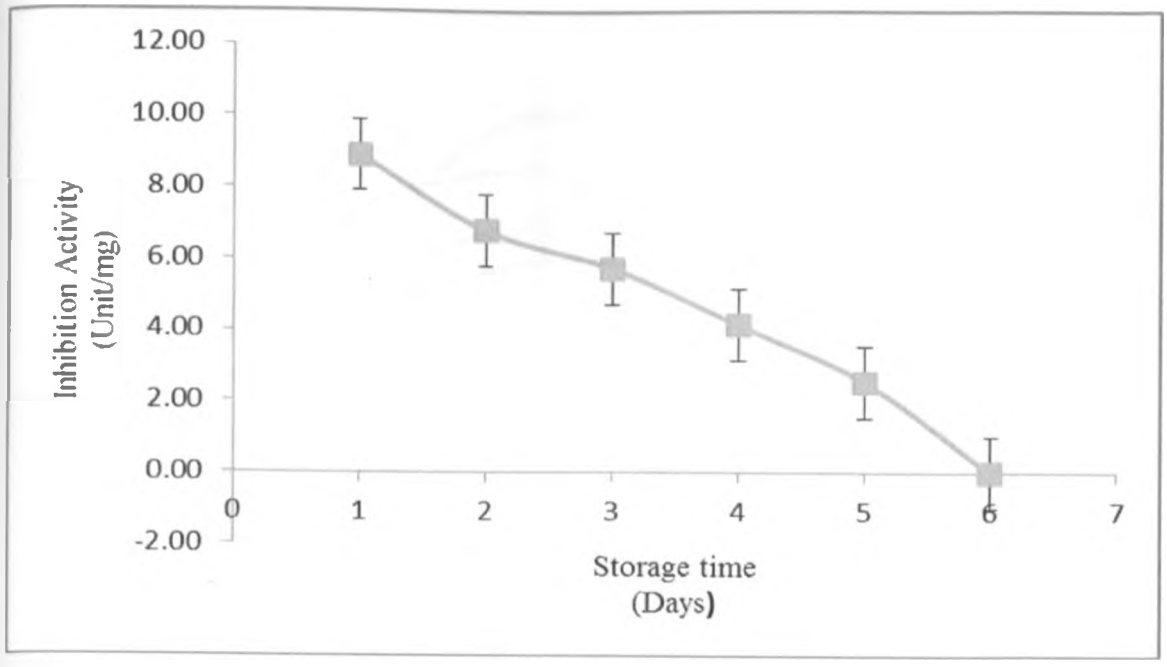

Figure 4: Effect of storage time (days) on inhibition activity of alpha amylase of methanolic extract. Data represents mean ± S.D. (n=3).

At the beginning of the storage period, the methanolic extract of the dried powder exhibited an inhibitory activity of 9.71 %. Over the 12 day storage period, there was a loss of inhibitory activity approximately by half (40.9 %) by the 3rd day of storage which represented the mid-point in one year.

4.5 RAT FEEDING TRIALS

4.5.1 Oral Glucose Loading Animal Model

The effect of different treatments on blood sugar levels over a period of time ranging from 0 to 2 hours is shown in figure 9. The onset of Charantin was noted after 30 minutes, which was when the glucose levels peaked in all treatment groups.

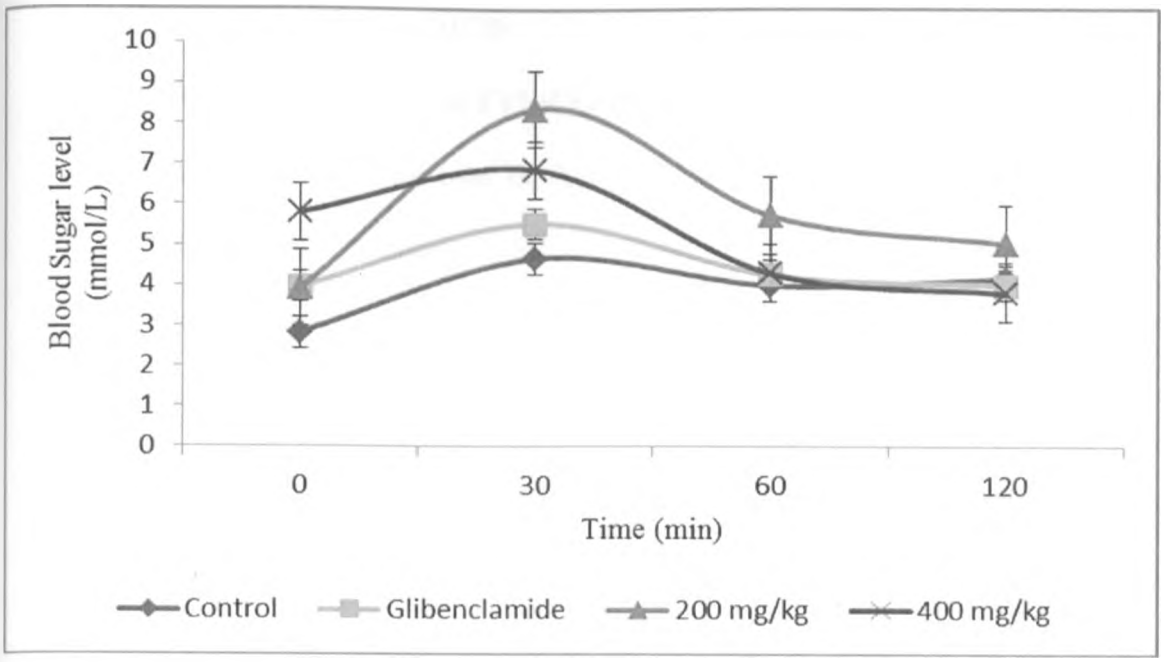

Figure 5: Effect of glucose (2mg/kg) in absence and presence of treatment regimes on glucose levels of normal rats. Data represents mean ± S.D. (n=3).

There was no significant difference in the glucose lowering effect of the control and the treatments ($p<0.05$). However, the contrast results from the K-matrix revealed that the treatment at the level of 400 mg/kg significantly ($p< 0.01$) affected blood glucose levels (0.072).

CHAPTER FIVE: DISCUSSION

5.1 LOCAL USES OF THE BITTER GOURD

The survey on the local uses of the bitter gourd revealed that although the fruit was readily grown and available in local markets, a majority of those familiar with the fruit were members of the Asian community and those allied to them, in this case vendors of the bitter gourd. Based on this, the recipes available were largely of Asian origin.

Its benefits, however, were well known and unanimously agreed upon, especially regarding its strong antidiabetic activity. Blending the fruit into a juice and consuming it when fresh was commonly stated as the best way to prepare it for use by a Diabetic person. The amounts in which it could be consumed varied from one respondent to the other and therefore there was no standard dosage that could be pinpointed. No mention was made on its use as a dried powder.

5.2 IN VITRO ANTIDIABETIC ACTIVITY

5.2.1 Moisture Content of bitter gourd samples

Drying removes the moisture from the food so that bacteria, yeast and mould cannot grow and spoil food. Drying also slows down the action of enzymes, but doesn't inactivate them. However, blanching, a necessary step for preparing vegetables for drying stops the storage enzyme action which could cause loss of colour and flavour during drying and storage (Harrison and Andress, 2012).

Vegetables are usually dried till brittle, hence only 10 % of moisture will remain, ensuring no growth of microorganisms. Hence the moisture content of 8.703 % achieved in this study is sufficient enough to ensure a long and stable shelf life.

5.2.2 Yield of Extracts

The aqueous and methanolic extraction was carried out according to the method by Virdi et al. (2003), resulting in a yield of 8.6 g per kg of dried fruit (0.86%) and 154.6 g per kg of dried fruit (15.47 %) respectively. These values differed from those reported by the same authors; 4.1 and 5.6 % respectively.

5.2.3 α-Amylase Inhibition Activity

Alpha-amylase is one of the main enzymes in man that is responsible for the breakdown of starch to more simple sugars thus the inhibitors of this enzyme can delay the carbohydrate digestion and reduce the rate of glucose absorption. Consequently, postprandial rise in blood glucose is decreased. Hence, they have long been thought to improve glucose tolerance in diabetic patients (Kwon et al., 2006).

The methanolic extract exhibited a dose-dependent increase in α-amylase inhibitory activity while the aqueous extract exhibited a decreasing trend of inhibition activity.

The decreasing inhibitory activity of aqueous extract is in contrast to studies whose results showed the highest hypoglycaemic activity in the aqueous extract when compared to methanol, chloroform (Virdi, 2003) and dried fruit powder (Srivastava, 1993). This result may be attributed to the property of the three main antidiabetic compounds of the bitter gourd, namely Charantin, polypeptide P and alkaloids, being soluble in organic

solvents such as ethanol and methanol, but not in water (Wikipedia, 2012; PatentStorm LLC, 2012; El- Said and Al-Barak, 2011).

5.2.4 Sensory Evaluation of Dried Powder

The attributes of taste, aroma and overall acceptability were used to represent the powder's sensory panel. The powder, in three different amounts, was scored highly based on the attributes. However, in terms of taste and aroma, it was not clear which amount was favoured best based on the fact that there lacked any significant difference in the overall acceptability among the three levels.

5.3 ACCELERATED SHELF-LIFE OF DRIED POWDER

5.3.1 Changes in the Antidiabetic Activity of Stored Samples

The dried *M. charantia* powder can be stored in sealed storage containers for approximately 6 months at room temperature (25 °C) before losing its α-amylase inhibitory activity by half. This becomes advantageous in situations where diabetics are unable to access the fresh product. Its shelf life when dried is also considerably longer than the fresh matter, because the fresh juice of the fruit needs to be ingested immediately after extraction.

5.4 RAT FEEDING TRIALS

5.4.1 Performance of Bitter Gourd Powder in Reducing Blood Glucose in Rats

The activity of the powder at the dosage of 400 mg/kg may be comparable to glibenclamide, a fact deemed as so because there was no significant difference in the blood glucose levels of the two groups at the end of the two hour study period,

confirming that the activity of the two treatments work with a similar efficiency (Virdi et al., 2003).

The reduction of blood sugar to normal levels by the powder in glucose loaded animals may show the possibility of the drug acting by increasing the glucose tolerance in diabetes (Leatherdale, 1981).

CHAPTER SIX: CONCLUSION AND RECOMMENDATIONS

6.1 CONCLUSION

It was observed that the knowledge on the uses of the bitter gourd fruit was held mainly by people from the Kenyan Asian community. However, it was highly recommended for use by diabetics (Type II) due to its blood sugar lowering effect; a fact known by persons other than the main users.

The antidiabetic assay (3-5dinitrosalicylic acid assay) showed that the methanolic extracts of *M. charantia* contain more antidiabetic activity than the aqueous extract due to the solubility of its antidiabetic compounds charantin and alkaloids in organic solvents.

The dose 400 mg/kg was found to have significant effect on blood sugar levels, hence when extrapolated to a dose usable by adults, an amount of 30 gms (1 tablespoon twice a day) would be suited to effectively lower blood glucose in an adult. This amount would be suitable going by the sensory evaluation scores for its overall acceptability. This should be ingested with water 15 minutes before a main meal. When extrapolated from the dose (30 gms of powdered bitter gourd), the required amount in fresh matter (blended juice) will be 330mls.

Indeed, the study successfully achieved its goal of developing powdered bitter gourd and proved its efficacy in significantly reducing blood sugar levels in this state; hence it would greatly be suited for use as a food agent in the management of diabetes.

Its stable antidiabetic activity when under storage proves that the powder possesses a long shelf-life hence its suitability for persons without storage facilities. Communities

can also be shown how to prepare, store and ingest the powder for maximum hypoglycaemic effects.

6.2 RECOMMENDATIONS

The following recommendations are made, based on the findings of this study:

The product can be stored at room temperature (25 °C) and ingested before a main meal in order to maintain maximal product antidiabetic activity.

It is important that procedures regarding induction of diabetes in an animal model should be standardized in view of the use of local rat breeds, preparation and administration of the diabetogenic drug under local environmental conditions, in order to achieve a stable state of diabetes.

It would be worthwhile to investigate the influence on the hypoglycaemic activity of the bitter gourd when used in conjunction with oral hypoglycaemic drugs – metformin and glibenclamide.

Further experiments should be carried out to determine quantities that would cause toxicity in important organs such as the liver and the kidney.

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APPENDICES

APPENDIX 1- KEY INFORMANT INTERVIEW GUIDE

1. What is the vernacular name of the bitter gourd?
2. Where do you access it?
3. How is it used in your community?
4. How frequently is it consumed?
5. How is it prepared (recipe)?
6. In what way is it used in the management of Diabetic patients?
7. How much success can you associate with its use?
8. What other food based approaches are employed in managing Type II Diabetes?
9. How does the bitter gourd compare to these?
10. Give reasons you can attribute to the success or failure of the bitter gourd in effectively lowering blood sugar levels.

APPENDIX 2 – SENSORY EVALUATION QUESTIONNAIRE

SENSORY ANALYSIS BY UNIVERSITY OF NAIROBI PANELLISTS

Name _____ Date _____

Instructions

Here are three samples for evaluation. Sprinkle onto your food and mix thoroughly. Please evaluate them for, taste, aroma and overall acceptability, using the intensity scale given below.

1= dislike very much
2= dislike moderately
3= dislike slightly
4= neither like nor dislike
5= like slightly
6= like moderately
7= like very much

Sensory Attribute	Amount (gm)		
	5	10	15
Taste			
Aroma			
Overall Acceptability			
Mean Score			

Comments:

APPENDIX 3

MALTOSE STANDARD CURVE