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HYPOGLYCEMIC EFFECTS OF SOME KENYAN PLANTS USED TRADITIONALLY IN THE MANAGEMENT OF DIABETES MELLITUS IN GACHOKA DIVISION, MBEERE DISTRICT

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A thesis submitted in partial fulfillment of the requirement for the award of the degree of Master of Science (Biotechnology) of Kenyatta University.

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*Hypoglycemic effects
of some Kenyan plants*



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Declaration

This thesis is my original work and has not been presented for a degree or any other award in any other university or any other institution.


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Dedication

I dedicate this work to my parents Samuel Karugu and Margaret Karugu whose love and support have made me what I am today. To my brothers Jim Murithii and Martin Mugendi and sister Diana Nyakio who have always been here for me.

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Abstract

Diabetes mellitus is a chronic disorder caused by inherited and/or acquired deficiency in production of insulin by the pancreas or by the ineffectiveness of the insulin produced by the target cells. Diabetes mellitus is characterized by high levels of glucose in blood, which in turn damages many of the body systems particularly the blood vessels and nerves. There are two forms of diabetes mellitus: type I diabetes mellitus and type II diabetes mellitus. Most conventional therapies for the management of type 2 diabetes include oral hypoglycemic drugs, exercise, diet and physical intervention therapies such as Acupuncture. Insulin is used in the management of type 1 diabetes mellitus. Insulin and oral hypoglycemic drugs are expensive and have numerous side effects. Through ages different communities have used medicinal herbs for diabetes mellitus management. Today herbal remedies are gaining popularity because the efficacy of conventional medicine is on the wane. This study was designed to bioscreen 7 aqueous medicinal plant extracts traditionally used to manage diabetes mellitus and assess their safety. Ethnobotanical and pharmacological information on the seven plants was gathered from the traditional healers. The plants collected from Mbeere district of Eastern province were *Caesalpinia volkensii*, *Vernonia lasiopus*, *Carissa edulis*, *Ficus sycomorus*, *Kleinia squarrosa*, *Azadirachta indica* and *Helichrysum odoratissimum*. All of them showed appreciable degree of hypoglycemic activity. Analysis of these plants for presence of trace elements showed that they contained varying amounts of Magnesium, Iron, Nickel, Copper, Zinc, Strontium, Molybdenum, Lead, Manganese, Chromium and Vanadium. Nickel and Strontium were present in one plant extract, two plant extracts had Manganese, and four plant extracts had Molybdenum. Chromium and Copper were present in six plant extracts and all the seven plants contained Iron, Lead and Magnesium. All plant extracts had undetectable quantities of Vanadium. Of the phytochemicals tested in the seven plants extracts, one plant had bound anthraquinones, two had alkaloids, sterol and triterpenes; three had saponins, four had flavonoids, and five had flavonols, flavones, chalcones and tannins. Free anthraquinones were not present in any plant extract. Trace elements and phytochemicals are associated with both the blood glucose lowering effect and toxicity. Toxicity of single plant extracts is reduced by the practice of using a combination of different plants extracts by the traditional healers. The study has established that the plants under study are effective and safe as antidiabetic medicines.

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CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

Diabetes mellitus is a chronic endocrinologic disorder caused by inherited and/or acquired deficiency in production of insulin by the pancreas or by the ineffectiveness of the insulin produced. It is characterized by high blood levels of glucose, which in turn damages many of the body systems particularly the blood vessels and nerves (Chen, 1998; WHO, 2003). This is caused by disturbances in the regulatory systems responsible for the storage and utilization of the chemical energy from food. This includes the metabolism of carbohydrates, fats and proteins resulting from defects in insulin secretion, insulin action, or both (Shillitoe, 1988; Votey and Peters, 2004).

According to WHO, more than 190 million people suffer from diabetes mellitus worldwide. The disease incidence is increasing rapidly and it is estimated that the figure will double by the year 2025. Most people with diabetes in developed countries will be aged 65 years or more by year 2025 yet, in developing countries the affected age bracket will be in the 45-65 year and in their most productive years (WHO, 2003). It is expected that the prevalence of diabetes will continue to increase in Africa and Asia as a result of changes in lifestyles and urbanization (Diabetes Control and Complications Trial Research Group, 1993). 49.0 million people suffer from the disease in South-East Asia; India alone accounts for almost a quarter of all patients in this region, with an estimate of 15 million people (Agrawal, 2004).

In the European continent an estimated 32.2 million patients suffer from diabetes; over a million of the cases being from United Kingdom alone (Effective Health Care, 1999). This figure accounts for over 2% of the UK population who suffer from the disease (Calman, 1998). Demographically, the Northern American continent has approximately 21.4 million. Latin America has an estimated 12.6 million diabetic patients (Harris *et al.*, 1987). In the United States diabetes mellitus is the third leading cause of death after heart disease and cancer and it affects approximately 17 million adults (Voet and Voet, 1995; Collins, 2002). In Australia it is a disease of modern society and it is estimated that 700,000 people are diabetic (Murtagh, 1999). In 2000 there were 7.5 million cases of diabetes in Africa and the figure is expected to rise to around 18.2 million by the year 2030 with about 190,000 sufferers from Kenya alone (Mngola, 2004). All African countries are struggling to care for a large number of diabetic patients, yet more than 80% of the cases are undiagnosed (Mngola, 2004). In Kenya the prevalence rate of diabetes is estimated at 3-10 % and 15% of this are people below 30 years. Diabetes affects up to 10 % of Kenyans, a percentage that could be higher as most cases of type 2 diabetes are diagnosed many years after onset (Njenga, 2005).

The effects of diabetes mellitus include long-term damage and failure of various organs, progressive development of the specific complications such as retinopathy which leads to blindness, nephropathy which may lead to renal failure, and/or neuropathy which may cause foot ulcers, amputation, and autonomic dysfunction, including sexual dysfunction. People with diabetes are at increased risk of cardiovascular, peripheral vascular and cerebrovascular diseases. Several pathogenetic processes are involved in the

development of diabetes. These include destruction of β -cells of the pancreas that lead to decreased sensitivity to insulin action (WHO, 1999; Votey and Peters, 2004).

Economic aspects of diabetes and diabetes care currently attract considerable attention as the world diabetes epidemic takes hold and the healthcare activities of countries come under pressure to accomplish more within constrained resources (Sobngwi *et al.*, 2001). Diabetes mellitus is a very expensive disease and has profound implications in terms of long-term microvascular and macrovascular complications and their associated cost. These complications reduce both life expectancy and quality of life (Ashcroft and Ashcroft, 1992; Collins, 2002; Votey and Peters, 2004).

Diabetes mellitus appears in two forms: type 1 diabetes mellitus or insulin dependent diabetes mellitus (IDDM) and type 2 or non- insulin dependent diabetes mellitus (NIDDM) (Voet and Voet, 1995). Type 1 is also referred to as juvenile-onset diabetes, occurring frequently in the early teen years in many patients. Type 1 diabetes mellitus is an inherited defect of the immune system triggered by environmental stimuli. The disease is characterized by the presence of autoantibodies to the pancreatic β -cells that produce insulin. The β -cells are inflamed and destroyed in this disease (Voet and Voet, 1995). Type 2 diabetes mellitus is a metabolic disorder resulting from the body's inability to produce sufficient, or to properly use, insulin. Patients with type 2 diabetes mellitus have either relatively low insulin production or insulin resistance, and occasionally require insulin administration (WHO, 1999).

Type 1 diabetes mellitus requires treatment with insulin injections, which involves injecting insulin under the skin in the fat for it to get absorbed into the blood

stream where it can then access all the cells of the body, which require it. The cost of insulin in Kenya is very high with the average cost of insulin vials being Ksh 1,500 to 2,000, which is out of reach of the low-income earners (Foster, 1974; Baron *et al.*, 2002; Njenga, 2004)). In type 2 diabetes mellitus, oral hypoglycemic drugs are used to control hyperglycemia.

In developing countries, traditional herbal medicines have continued to play an important role side by side with modern medicine especially in primary health care in poorer or rural areas. Herbal preparations constitute valuable natural resources from which chemicals of great potential for agriculture and medicine are found (Seneader, 1985).

Plant derivatives with presumed hypoglycemic properties have been used in folk medicine and traditional healing systems globally such as Native American Indian, Jewish Chinese East Indian and Mexican (Yaniv *et al.*, 1987; Covington, 2001). Many modern pharmaceuticals used in conventional medicine have also natural plant origins. Among them metformin, derived from the flowering plant, *Galega officinalis*, is a common traditional remedy for diabetes (Pandey *et al.*, 1995; Oubre *et al.*, 1997). Similarly, the use of vitamin and mineral supplements to treat or prevent primary or secondary disease is of increasing interest (O'Connell, 2001)

There are other forms of diabetes that include gestational diabetes, brittle diabetes, unstable diabetes and ketosis prone diabetes. Patients suffering from the ketosis prone diabetes have a classic symptom of ketones present in blood and urine (Guthrie and Guthrie, 1999)

Greater prevalence of diabetes complications has been reported in populations of African origins compared to Caucasians; this is because of poor compliance and or difficult access to appropriate care and affordability of treatment in difficult socioeconomic environments (Cowie, 1993; Goldschmid *et al.*, 1995; Musey *et al.*, 1995; Delamater *et al.*, 1999).

1.2 Epidemiology, Prevalence and Incidence of Diabetes Mellitus

Epidemiological studies show that diabetes mellitus develops from a complex interaction between environmental and genetic factors. For example, the offspring and siblings of diabetic parents are more likely to develop diabetes than those of nondiabetic parents; the offspring of two diabetic parents is more likely to develop diabetes than an offspring having only one diabetic parent; the lower than expected frequencies of diabetes in identical twins and offspring and siblings of diabetics are suggestive of the importance of environmental factors in the expression of the genetic component for diabetes. A second event such as a viral infection or a disturbance of the immune system occurring early in life is postulated to trigger type 1 diabetes in genetically susceptible individuals. Type 2 occurs without such events though its expression is modulated by factors such as obesity. Inheritance plays a more important role in development of type 2 diabetes than type 1 diabetes (Kaplan and Pesce, 1996).

There is a global trend towards increases in the incidence and prevalence of diabetes mellitus in African populations (King *et al.*, 1998). Global estimates of the number of people with diabetes in Africa was approximately 3 million in 1994 and is expected to increase 2-3 fold by the year 2010 (Amos *et al.*, 1997). The prevalence of

diabetes in African communities is increasing with ageing of the population and lifestyle changes, which are associated with rapid urbanization and westernization (King *et al.*, 1999). Urban residents have a 1.5-4 fold higher prevalence of diabetes compared to their rural counterparts. Urban lifestyles in Africa are characterized by changes in diet where subjects consume high levels of refined sugars and saturated fat and reduction in fiber intake coupled with reduced physical activity (Sharma *et al.*, 1996; Gill *et al.*, 1997; Mennen *et al.*, 2000).

Diabetes mellitus displays a geographic, ethnic and racial prevalence especially in type 2 diabetes mellitus. The disease is more prevalent among Hispanics, Native Americans, African Americans and Asians Pacific Islanders than in whites. The Pima Indians living in the Arizona in the United States of America have the highest rate of type 2 diabetes mellitus in the world. South Eastern Asians as well as the Australian Aborigines are at a higher risk of developing non-insulin dependent diabetes mellitus when compared to the Caucasians (Osei, 1995; Gill *et al.*, 1997; Votey and Peters, 2004). Type 2 diabetes mellitus, is the predominant form (70-90%); this is due to high urban growth rate, dietary changes, and reduction in physical activities and increasing obesity. It is estimated that the prevalence of diabetes is due to triple within the next 25 years.

Type 1 diabetes mellitus also displays a geographical and regional prevalence. Estimates show that 5.3 million people live with type 1 diabetes worldwide, of which 395,000 or 7.4% are children. The disease is common in areas near the equator (Leslie and Gale, 1995). Consumption of large quantities of cow's milk during childhood may increase the risk of developing type 1 diabetes in children who are already genetically

susceptible to the disorder. Children who have siblings with diabetes are more than five times as likely to develop the autoimmune disorder if they drink more than half a liter (about three glasses) of cow's milk a day, compared with children who drink less milk (New York Health Reuters, 2000). Type 1 diabetes tends to have fewer tendencies to have other family members affected with diabetes than type 2. In the first large family study of diabetes, less than 4% of parents and 6% of siblings of a person with diabetes also had diabetes. The incidence of diabetes mellitus is equal in females and males in all populations but it is greater for blacks than whites. It is estimated that slightly more than 218,000 persons develop type 1 diabetes worldwide annually. Of these, 86,400, or 40% are children. The Europeans contribute 60,000 new cases annually, while the Southeast Asian region contribute 45,000 new cases, followed by the North American region with 36,000 new cases annually. The African region contributes the lowest number of cases, 6,900 persons annually. The proportion of children among the new cases ranges from 29% in the European Region to 54% in the African Region, reflecting the combined effect of differences in age structure and incidence levels (Kaplan and Pesce, 1996).

Type 2 diabetes is becoming increasingly common because more people are living longer as diabetes prevalence increases with age. It is also being seen more frequently in younger people in association with the rising prevalence of childhood obesity. Although type 2 diabetes still occurs most commonly in adults aged 40 years and older, the incidence of disease is increasing more rapidly in adolescents and young adults than in other age groups (Votey and Peters, 2004).

1.3 Types of Diabetes Mellitus

1.3.1 Type I Diabetes Mellitus

Type I diabetes or insulin dependent diabetes mellitus (IDDM) is a chronic autoimmune disease in which the body's own immune system attacks the β -cells in the Islets of Langerhans of the pancreas, destroying them or damaging them sufficiently to reduce insulin production. It is thought that the autoimmunity against β -cells is induced in a susceptible individual by a foreign antigen, such as a virus that immunologically resembles some β -cell component (Voet and Voet, 1995).

Environmental factors, such as a virus, initiates the process of beta cell destruction in genetically susceptible individuals. This external influence precipitates an inflammatory response in the pancreas known as insulinitis. Activated T-lymphocytes infiltrate the islet cells in the pancreas and cause β -cell destruction via localized release of cytokines. Cytotoxic amounts of nitric oxide and reactive oxygen intermediates are also released, contributing to free radical damage to the β -cells. The initial steps in free-radical induced islet cell death involve breaks in DNA strands and the activation of the enzyme poly (ADP-ribose) polymerase (PARP). PARP is involved in DNA repair and consumes large amounts of NAD^+ in the process. The depletion of intracellular NAD^+ pools leads to islet cell death (Dahlquist, 1994).

Infectious viral agents may also stimulate the immune system to attack its own cells. Viruses that trigger the immune system may also penetrate the β -cells and cause their destruction leading to a decrease in insulin secretion by the pancreas. Other than viruses, other environmental stimuli may stimulate the immune system to attack the islets

of langerhan hence their destruction. This attack on the β -cells leads to a decline in insulin production (Lucassen and Bell, 1995; Kaplan and Pesce, 1996; Weiss, 1997). Type I diabetes mellitus results from the gradual disappearance of insulin production. Although age-related, type 1 diabetes mellitus is not confined to the young or even the middle aged but may occur rarely in the elderly. The sex distribution is equal (Shillitoe, 1988; Greens, 1995).

Type I diabetes has a genetic component which must be present for susceptibility to occur. Although the exact mechanism is unclear, transmission is believed to be autosomal dominant, recessive or mixed. If a first-degree relative has the disease, the child has a 5-10 % chance of developing type-I diabetes. The susceptibility gene resides on the short arm on the sixth chromosome, either within or in close proximity to the major histocompatibility complex, that is, the HLA region. The major alleles conferring risk of type I are HLA-DR3, HLA-DR4, HLA-Dw3, HLA-Dw4, HLA DQ, HLA DP, HLA-B8 and HLA-B15 (Hawkes 1997). However, no single HLA allele or combination is specific for susceptibility to type I. Type I is strongly associated with particular HLA-DQ-encoded heterodimers. On the other hand, some alleles of the major histocompatibility complex confer protection against the development of type I. These are HLA-DR2 and HLA-DQ B1 and they appear to have dominance over susceptibility alleles (Nepom and Ehrlich, 1991).

1.3.2 Type II Diabetes Mellitus

Type II diabetes mellitus is a syndrome characterized by disordered metabolism of carbohydrate and fat. This leads to hyperglycemia with fasting plasma glucose level

>126 mg/dL (Diabetes Care, 1997). Insulin resistance and a relative β -cell defect are the underlying pathological problems leading to hyperglycemia (Shillitoe, 1988; Collins, 2002)

The disease is characterized by peripheral insulin resistance in insulin targeting tissues such as the skeletal muscle and adipocytes with an insulin secretory defect and deficiency of insulin receptors (Votey and Peters, 2004). The type 2 diabetes mellitus is the most common form of diabetes and it accounts for over 90% of the diagnosed cases of diabetes (Gursche and Rona, 2000). Type 2 diabetes usually develops in middle age or later. This tendency to develop later in life has given rise to the term "adult onset diabetes" (Kaplan and Pesce, 1996). The typical type 2 diabetes mellitus occurs in obese individuals with a genetic pre-disposition for this condition although the genes responsible for the disease causation are carried on different chromosomes from the type 1 diabetes. Type 2 diabetes mellitus shows strong familial aggregations. Family studies show that the genetic component in causing diabetes mellitus is relatively strong. Several genes have been suggested as markers for type 2. Obesity is associated with insulin resistance (Shillitoe, 1988; Frogel, 1992; Voet and Voet, 1995; Votey and Peters, 2004).

NIDDM is a growing problem among the developing countries (Moshi *et al.*, 2000). There is probably a social class bias in the prevalence of the type 2 diabetes with the disorder being more common in socially disadvantaged groups (Shillitoe, 1988). This is due to the changing socio-economic factors, which in turn affect the dietary and living conditions of the people (Moshi *et al.*, 2000)

The genetic factor in addition to some environmental factors such as aging, excess caloric intake followed by deficient caloric expenditure and obesity may result to insulin resistance. This makes the body produce more insulin to overcome the resistance. The resistance to the action of insulin is associated with excessive production of glucose by the liver and impaired glucose utilization by peripheral tissues especially the muscles. Overtime, chronic stimulation of insulin secretion diminishes and the islet β -cell reserve is exhausted. Clinical diabetes is as a result of a state of absolute insulin deficiency. The production of more insulin to overcome resistance leads to either exhaustion of the β -cells resulting in insulin deficiency or a condition known as glucotoxicity. Sugar in high amounts may be toxic to cells of the body. The toxicity damages the β -cells and a decline in insulin secretion ensues (De Fronzo, 1987; Seely and Olesky, 1993; Olesfsky, 1999; Guthrie and Guthrie, 1999).

1.3.3 Other Forms of Diabètes

1.3.3.1 Brittle diabetes

Brittle diabetes is frequently characterized by very frequent and extreme oscillations between hypo and hyperglycemia (Elber *et al.*, 1997). It is an uncommon complication of type 1 diabetes but the seriousness of the complication and demands on the health care systems warrants aggressive intervention. Brittle diabetes is always secondary to a specific, identifiable etiology. There are many different causes of brittle diabetes, but the most common is a psychological abnormality (Elber *et al.*, 1997).

1.3.3.2 Gestational Diabetes Mellitus (GDM)

Gestational diabetes mellitus (GDM) is defined as any degree of glucose intolerance with onset of pregnancy (Votey and Peters, 2004). It occurs when the pregnant woman's body cannot produce enough insulin, resulting in high blood sugar. GDM affects an estimated 2-3 % of pregnant women (WHO, 1999; Wikipedia the free encyclopedia, 2004). It is estimated that 39 % of women with gestational diabetes manifest type 2 diabetes mellitus later in life (Kaplan and Pesce, 1996).

This disease also leads to higher rates of cesarean delivery and chronic hypertension (Votey and Peters, 2004). Generally a test for gestational diabetes is undertaken between the 24th and 28th week of pregnancy. Often, gestational diabetes can be managed through a combination of diet and exercise. If that is not possible, it is treated with insulin, in a similar manner to diabetes mellitus (Guthrie and Guthrie, 1999; WHO, 1999).

Pregnancy may favor the occurrence or aggravation of diabetic retinopathy and neuropathy in patients with pre-existing diabetes (Boivin *et al.*, 2002). The mother is also at a risk of developing toxemia and eclampsia (Guthrie and Guthrie, 1999). Fetuses from pregnancies with gestational diabetes have a high risk of macrosomia, asphyxia, neonatal hypoglycemia and hyperinsulinaemia, excessive fat accumulation, insulin resistance, pancreatic exhaustion and possible risk of child and adult obesity and type 2 diabetes mellitus later in adult life (Glueck *et al.*, 2002).

The pathophysiology of gestational diabetes includes insulin resistance and decreased insulin secretion (Boivin *et al.*, 2002). Although glucose tolerance normalizes

shortly after pregnancy, with gestational diabetes in the majority of women the risk of developing type 2 diabetes is especially high (Vambergue *et al.*, 2002). The risk factors involved in gestational diabetes include a family history of type 2 diabetes. Maternal age is also a risk factor, the risk increasing with the age of the woman (Vambergue *et al.*, 2002).

1.3.4 Causes of Diabetes Mellitus

Diabetes mellitus can be caused by use of drugs that are toxic to the β -cells and cause drug-induced diabetes. These drugs include alloxan, streptozocin, dilantin, thiazide, pentamidine and α -interferon therapy. High doses of glucocorticoides, such as steroids, some cancer chemotherapeutic agents especially L-asparagines, antipsychotics and mood stabilizers (phenothiazines) are also known to cause diabetes mellitus. Many drugs can impair insulin secretion. They elevate blood sugar level through various mechanisms (WHO, 1999).

These drugs may not, by themselves, cause diabetes but they may precipitate diabetes in persons with insulin resistance (Phelps *et al.*, 1989; O'Byrne and Feely, 1990; Pandit *et al.*, 1993; Votey and Peters, 2004). Certain toxins such as Vacor a rat poison and pentamidine can permanently destroy pancreatic β -cells (Gallanosa *et al.*, 1981; Esposti *et al.*, 1996; WHO, 1999). Hormones can also impair insulin action. Such hormones include thyroid hormone, α -adrenergic agonists, β -adrenergic agonists, growth hormone, cortisol, glucagons and epinephrine, which antagonize insulin action. Diseases associated with excess secretion of these hormones can cause diabetes, for instance, acromegaly, Cushing's syndrome, glucagonoma and pheochromocytoma (Phelps *et al.*,

1989; Mac Farlane, 1997; WHO, 1999; Wikipedia the free encyclopedia, 2004). Diseases of the exocrine pancreas and processes that diffusely injure the pancreas can cause diabetes. Acquired processes include pancreatitis, trauma, infection, pancreatic carcinoma, and pancreatectomy (Larsen *et al.*, 1987; Gullo *et al.*, 1994). With the exception of cancer, damage to the pancreas must be extensive for diabetes to occur. However, adenocarcinomas that involve only a small portion of the pancreas have been associated with diabetes. Somatostatinoma, and aldosteronoma-induced hypokalaemia, can cause diabetes, at least in part by inhibiting insulin secretion (Conn, 1965; Krejs *et al.*, 1979).

Certain viruses have been associated with β -cell destruction. Diabetes occurs in some patients with congenital rubella viruses that include Coxsackie B, cytomegalovirus and other viruses (Forrest, 1971). Adenovirus and mumps have been implicated in inducing the disease (Pak *et al.*, 1988; King *et al.*, 1998). Many genetic syndromes are accompanied by an increased incidence of diabetes mellitus. These include the chromosomal abnormalities of Down's syndrome, Klinefelter's syndrome and Turner's syndrome. Wolfram's syndrome, an autosomal recessive disorder characterized by insulin-deficient diabetes and the absence of β -cells at autopsy, also causes hyperglycemic states and if the glycemic status is prolonged it leads to permanent diabetes. Other genetic syndromes sometimes associated with diabetes are Friedreich's ataxia, Huntington's chorea, Lawrence-Moon-Biedel syndrome, myotonic dystrophy, porphyria and Prader-Willi syndrome (Barrett *et al.*, 1995).

1.3.5 Symptoms of Diabetes Mellitus

Type 2 diabetes mellitus has a slow onset often taking years, but in type 1, particularly in children, onset may be quite fast within weeks or months. Early symptoms of type 1 diabetes are polyuria, polydipsia and consequent increased fluid intake. There may also be weight loss despite normal or increased eating, increased appetite and irreducible fatigue. These symptoms may also manifest in type 2 diabetes in patients who present poorly controlled diabetes. Thirst develops because of osmotic effects, that is, sufficiently high glucose above the renal threshold in the blood is excreted by the kidneys but this requires water to carry it and causes increased fluid loss, which must be replaced. The lost blood volume will be replaced from water held inside body cells, causing dehydration (WHO, 1999).

Another common presenting symptom is altered vision. Prolonged high blood glucose causes changes in the shape of the lens in the eye, leading to blurred vision. Especially dangerous symptoms in diabetics include the smell of acetone on the patient's breath (a sign of ketoacidosis), a rapid, deep breathing, and an altered state of consciousness or arousal. Hostility and mania are both possible, as is confusion and lethargy. The most dangerous form of altered consciousness is the so-called "diabetic coma" which produces unconsciousness. Early symptoms of impending diabetic coma include polyuria, nausea, vomiting and abdominal pain, with lethargy and somnolence, a later development, progressing to unconsciousness and death if untreated. Irritability, skin infections or sores that take long to heal and vaginal infections in women are also some common symptoms of diabetes mellitus (Dierckx *et al.*, 1996; WHO, 1999). These

symptoms affect the quality of life of an individual and when severe can lead to a decrease in work performance in adults and an increase in the number of falls in the elderly (Davison, 1991).

1.3.6 Complication of Diabetes Mellitus

Diabetes mellitus is a systemic disease that affects every organ of the body. Cardiovascular and renal lesions are the most common abnormalities leading to death.

1.3.6.1 Diabetic retinopathy

Diabetic retinopathy is a leading cause of blindness and visual disability (WHO, 2003). It is the most common cause of blindness in people of working age in industrialized countries (Williams, 1994). Up to 40% of people have some retinopathy when type 2 diabetes is first diagnosed (Diabetic Retinopathy Study Research Group, 1998). Twenty years after diagnosis, all of those with type 1 diabetes and 60% with type 2 diabetes have some degree of retinopathy (Diabetic Retinopathy Study Research Group, 1998). In diabetic retinopathy, small blood vessels in the retina (back of the eye) become blocked, swollen or leaky, causing edema, and new, fragile vessels grow haphazardly in the retina. This process continues for years without causing visual symptoms or visual impairment; during this period, retinopathy is only detected by eye examination. If it is left untreated, bleeding and scarring leads to progressive loss of vision (Early Treatment Diabetic Retinopathy Study Research Group, 1991; Kaplan and Pesce, 1996; Diabetic Retinopathy Study Research Group, 1998).

1.3.6.2 Diabetic ketoacidosis (DKA)

Diabetic ketoacidosis (DKA) is one of the severe complications of diabetes mellitus. DKA is a medical emergency and if left without prompt proper treatment, patients have substantial chance of death. Before the era of insulin, DKA was the leading cause of patient deaths (Beigelman, 1971; WHO, 1999; Wikipedia the free encyclopedia, 2004). DKA is characterized by high blood sugar, or hyperglycemia. The complication begins with physiologic stress that causes release of catecholamines, glucagon, and cortisol. This stress may be emotional or physical, although the most common cause by far is infection (e.g., pneumonia) (American Diabetes Association, 1995; Diabetic Retinopathy Study Research Group, 1998).

This complication is most common in type 1 diabetes mellitus where there is no circulating insulin. In situations where there is a severe deficiency in insulin levels, the body switches to fat metabolism, a mechanism which actually exists to protect the organs during periods of starvation, as glucose is not available to be taken up due to lack of insulin even though blood levels of glucose are high. Ketones are produced from fats, partly because the brain utilize ketones for energy as they can pass the blood-brain barrier. As the level of available glucose for the brain and other organs runs low (due to the persistent low levels of insulin despite the rising levels of serum glucose as a by product of the fat metabolism,) more and more fats are metabolized releasing more and more ketones (WHO, 1999).

Accumulation of these ketone bodies results in metabolic acidosis as pH buffers in the serum are used up. Rising levels of glucose and ketones increase the osmolality of

the serum. the hyperglycemic state initially encourages the patient's kidneys to produce more urine, causing the body to lose water and electrolytes such as potassium and phosphate, leading to dehydration and hypokalemia. Treatment consists of hydration to lower the osmolality of the blood, replacement of lost electrolytes, insulin to force glucose and potassium into the cells, and eventually glucose simultaneously with insulin in order to correct other metabolic abnormalities, such as elevated blood potassium (hyperkalemia) and elevated ketone bodies. Survival is dependent on how badly deranged metabolism is at presentation to a hospital, but the process is only occasionally fatal (Beigelman, 1971; Lehninger *et al.*, 1982; American Diabètes Association, 1995; Kaplan and Pesce, 1996)

1.3.6.3 Angiopathy

Angiopathy is one of the complications of diabetes mellitus, which occurs after the patient has had the disease for along time. It refers to the damage to linings (basement membranes) of blood vessels. There are two types of angiopathy; one is macro-angiopathy where fat and blood clots build upon the blood vessels and stick to the walls and block the flow of blood. The other type is micro-angiopathy where the walls of the small blood vessels become so thick and weak that they bleed and therefore leak protein and slow blood flow through the body. Cells such as those at the center of the eye do not get enough blood and may be damaged. Angiopathy increases the risk of coronary heart disease and stroke and can lead to retinopathy and nephropathy (Kaplan and Pesce, 1996; WHO, 1999).

1.3.6.4 Nephropathy

Diabetes is among the leading causes of kidney failure, but the frequency varies between populations and also due to the severity and duration of the disease (WHO, 2003). Nephropathy refers to the damage of the glomerulus and its associated capillaries. Capillary damage is caused by angiopathy and the result is a reduction in the filtering capacity of the kidneys. Proteinuria is the first sign of diabetes nephropathy (Kaplan and Pesce, 1996).

1.3.6.5 Hyperglycemia

Acute hyperglycemia, even when not associated with DKA, is harmful to the body because if the blood glucose level exceeds the renal threshold for glucose, an osmotic diuresis ensues, with loss of glucose, electrolytes, and water. Hyperglycemia impairs leukocyte function through a variety of mechanisms. As a result of high blood glucose levels patients with diabetes have a low resistance to illness, an increased rate of wound infection, and impaired wound healing (Gursche and Rona, 2000).

Management of hyperglycemia during medical illness and surgery is important because serious medical illness and surgery produce a state of increased insulin resistance. Blood glucose levels of 1000-2000 mg/L are maintained in medical and surgical patients with diabetes to prevent electrolyte abnormalities and volume depletion secondary to osmotic diuresis. This is also done to prevent the impairment of leukocyte function and wound healing that occurs when blood glucose levels are elevated (Votey and Peters, 2004).

1.3.6.6 Diabetic neuropathy

Diabetic neuropathy is the most common complications of diabetes mellitus and occurs in 50 % of diabetics (WHO, 2003). Acute hyperglycemia decreases nerve function and chronic hyperglycemia is associated with the loss of myelinate and unmyelinated fibers and loss of nerve conduction. The complication is recognized by a number of symptoms that include numbness, pain, tingling or burning sensations in the extremities, dizziness and double vision. Other symptoms are decreased gastric motility, erectile dysfunction, bladder dysfunction and impaired cardiac function (Kaplan and Pesce, 1996; Didier, 2000; WHO, 2003)

1.3.6.7 Diabetic Foot Disease

At some time in their life, 15% of people with diabetes develop foot ulcers associated with peripheral neuropathy and ischaemia, peripheral vascular disease and superimposed infections (Apelqvist *et al.*, 1993; Boulton *et al.*, 1995; WHO, 2003). Diabetic foot disease is one of the most costly complications of diabetes especially in communities with inadequate foot wear (WHO, 2003). Hyperglycemia is associated with mild defects in nerve conduction and the feet become insensitive. Neuropathy also leads to deformed foot secondary to tendon shortening which leads to decreased motility of the foot. The combination of foot insensitivity and foot deformities allows undue stress to small areas of the foot and promotes foot ulcers. Infection is a frequent complication of both vascular and neuropathic ulcers (Wheat, 1980).

Recurrence rates for diabetic foot ulcers are 35–40% over three years and 70% over five years (Apelqvist *et al.*, 1993). These ulcers can have serious consequences.

They are highly susceptible to infection, which may spread rapidly, causing overwhelming tissue destruction (Edmonds *et al.*, 1986). 5–15% of people with diabetic foot ulcers require lower extremity amputation, usually because of gangrene; foot ulcers precede 85% amputations in people with diabetes (Pecoraro *et al.*, 1990; Larsson, 1994). Up to two thirds of non-traumatic amputations are in people with diabetes whose ulcers have progressed to gangrene (Peacock *et al.*, 1985; Bild *et al.*, 1989; Laing *et al.*, 1991; Effective Health Care, 1999; WHO, 2003).

1.3.6.8 Metabolic disorders

Metabolic complications are more common in type 2 than in type 1 diabetes mellitus. They include non-ketotic hyperglycemic hyperosmolar coma (NKHHC) and hypoglycemia. NKHHC is most common in older patients with type 2 diabetes. It is a life threatening often fatal complication that occur spontaneously in persons with undiagnosed diabetes mellitus. It can also occur after long periods of uncontrolled hyperglycemia. The predisposing factors range from the use of potentially hyperglycemic inducing agents, surgical procedures, or acute or chronic diseases and infections. NKHHC is characterized by severe hyperglycemia (greater than 6000 mg/L), absence or right ketosis, plasma hyperosmolality and profound dehydration (Foster, 1974).

1.3.6.8.1 Hypoglycemia

Hypoglycemia occurs in both type 1 and 2 diabetes and arises as a result of poor management of the disease either from too much or poorly timed insulin or oral hypoglycemics or too much exercise, not enough food, or poor timing of either.

Aggressive use of insulin treatment to maintain normal glucose levels can increase the risk of hypoglycemia (Kaplan and Pesce, 1996).

Diabetics usually carry something sugary to eat or drink as these symptoms are rapidly reduced if treated early enough. Other ways of treating hypoglycemia include an injection of glucagon which causes the liver to convert its internal stores of glycogen to be released as glucose into the blood. Oral or intravenous dextrose is also given. In most cases, recovery is rapid and trouble free. Longstanding hypoglycemia require hospital admission to allow supervised recovery and adjustment of diabetic medications (WHO, 1999)

1.3.6.8.2 Arteriosclerosis

This is an occlusive vascular disease and it is the most common complication of type 2 diabetes. The lesions of arteriosclerosis represent a series of highly specific cellular and molecular responses that have many characteristics of an inflammatory disease (Steiner, 1981). These lesions occur in medium and large sized arterials and lead to ischemia of the heart, brain or extremities resulting in infarction, stroke or peripheral extremity ischemia. The arteries accumulate plaque slowly over the years. The patient remains asymptomatic, the process accelerates gradually until occlusion occurs and ischemia and tissue death results (Wheat, 1980).

1.3.6.8.3 Lactic acidosis

Lactic acidosis is a serious condition characterized by excessive accumulation of lactic acid and metabolic acidosis and this results from tissue hypoxia (oxygen deficiency) (Kaplan and Pesce, 1996). The hallmark of lactic acidosis is the presence of

tissue hypoxemia that leads to enhanced anaerobic glycolysis with increased lactic formation. In diabetes mellitus, lactic acidosis is seen in association with alcohol intoxication, phenformin therapy and ketoacidosis. Pyruvic acid is converted into lactic acid by lactic dehydrogenase (LDH) in the presence of the reduced nicotinamide dinucleotide (NADH), which in turn is converted into NAD. The reaction is reversible and involves LDH in both directions. The conversion of acetoacetate to β -hydroxybutyrate also requires the oxidation of NADH. Lactic acidosis results from decreased availability of NAD, which is secondary to lack of oxygen. The deficiency of NAD also impairs the conversion of β -hydroxybutyrate into acetoacetate. Thus lactic acidosis predisposes to accumulation of β -hydroxybutyrate. Like the accumulation of keto acids, lactate accumulation causes increased blood hydrogen ions and therefore low pH (Gabbay, 1975; Kaplan and Pesce, 1996).

1.3.6.8.4 Hyperosmotic coma

Hyperosmotic diabetic coma is another acute problem associated with improper management of diabetes mellitus. It has some symptoms in common with DKA, but of a different cause, and requires different treatment. With very high blood glucose levels (>300 mg/dl) water is osmotically driven out of the cells into the blood. The kidneys also dump glucose into the urine, resulting in concomitant loss of water, an increase in blood osmolarity. The osmotic effect of high glucose levels combined with the loss of water result in high serum osmolarity that the body cells become directly affected as water is drawn out. Electrolyte imbalance is also common. This combination of changes, if

prolonged, results in symptoms similar to ketoacidosis, including loss of consciousness. As with DKA, urgent medical treatment is necessary (WHO, 1999).

1.4 Diagnosis of Diabetes Mellitus

The diagnosis of diabetes should never be made on the basis of a single abnormal blood glucose value. At least one additional plasma/blood glucose test result with a value in the diabetic range is essential, either fasting, from a random (casual) sample, or from the oral glucose tolerance test (OGTT). If such samples fail to confirm the diagnosis of diabetes mellitus, it is advisable to maintain surveillance with periodic re-testing until the diagnostic situation becomes clear. In these circumstances, the clinician takes into consideration such additional factors as ethnicity, family history, age, adiposity, and concomitant disorders, before deciding on a diagnostic or therapeutic course of action (WHO, 1999).

Diabetes screening is recommended for many types of people at various stages of life or with several different risk factors. Many health care recommendations for adults recommend universal screening at age 40 or 50 years, and sometimes occasionally thereafter. Earlier screening is recommended for those with risk factors such as obesity, family history of diabetes, high risk ethnicity such as Hispanic, Indian American, African American, Pacific Island, and South Asian ancestry (WHO, 1999).

1.4.1 Functional Tests

1.4.1.1 Postprandial Plasma Glucose

Diabetes is more readily detected when the carbohydrates metabolic capacity is tested by stressing the system with a defined glucose load. Measurement of the rate at

which glucose load is cleared from the blood compared to the rate of glucose clearance in healthy persons detects any impairment in glucose metabolism. A meal high in carbohydrates is used as carbohydrate load though a 75g glucose drink is preferred over a meal. In postprandial plasma test, blood is drawn two hours after ingestion of the meal or drink. Levels of 1200 to 1400 mg/l are ambiguous and levels below 1200 mg/l are normal. This test is highly inaccurate because several variables are difficult to control and adjust. These include age, weight, previous diet, activity, illness, medication, time of the day that the test is conducted and the actual size of the glucose dose. When a meal is used as the load, the effective glucose load depends on the digestion of disaccharides and polysaccharides and their subsequent absorption from the intestinal tract (Kaplan and Pesce, 1996).

1.4.1.2 O' Sullivan Test

This test is used to detect gestational diabetes. A 50g load of glucose is given to a fasting patient and blood drawn after one hour. Gestational diabetes is suggested by plasma glucose levels above 1500 mg/l (Kaplan and Pesce, 1996).

1.4.1.3 Oral Glucose Tolerance Test

This test evaluates glucose clearance from the circulation after glucose loading under defined controlled conditions. The conditions include a minimum carbohydrate intake of 150g/day 3 days before the test among others. Daily carbohydrate intake less than this lowers carbohydrate intolerance. There should be an 8-16 hour fasting before testing. The patient should be active since inactivity decreases glucose tolerance. Exercise and emotional stress should be avoided. For most subjects 75g total glucose load is

sufficient. Blood samples are drawn at fasting and 1, 2 and 3 hours after ingestion of glucose. WHO suggests impaired glucose tolerance or diabetes when both fasting and 2 hour plasma glucose levels exceed 1390 mg/l, when the 2 hour value is 1400 to 2000 mg/l, impaired glucose tolerance is suggested and when the 2 hour value exceeds 2000 mg/l diabetes mellitus is suggested (Kaplan and Pesce, 1996).

1.4.1.4 Intravenous Glucose Tolerance Test

This test is used for persons with malabsorption disorders or previous gastric or intestinal surgery. A glucose load of 0.5g/kg body weight is used. Nondiabetics respond with a plasma glucose level of 2000 to 2500 mg/l. Discontinuations of the glucose loading decreases plasma glucose levels within about 90 minutes. Diabetics demonstrate plasma glucose levels above 2500 mg/l during administration of the load. On discontinuation of the loading, plasma glucose levels in diabetics also return to fasting levels after about 90 minutes (Kaplan and Pesce, 1996).

1.5 Management of Diabetes Mellitus

1.5.1 Conventional Therapy

1.5.1.1 Insulin

Until the 1950s, insulin therapy was the only treatment available for diabetes mellitus (Jahodar, 1993). Insulin is a polypeptide hormone that has the empirical formula $C_{254}H_{377}N_{65}O_{75}S_6$. It is synthesized in the endocrine pancreas by the beta cells of the pancreas otherwise known as islets of Langerhans (Kaplan and Pesce, 1996 ; <http://en.wikipedia.org/wiki/Insulin> 2004) The hormone is responsible for the regulation of carbohydrates and triacylglyceride metabolism. Insulin acts mainly on muscle, liver and

adipose tissue cells to stimulate synthesis of glycogen, fats and proteins while inhibiting the breakdown of these metabolic fuels (Voet and Voet, 2004). The stimulus for insulin secretion is high blood glucose. The pancreas normally secretes low levels of insulin, but the amount secreted into the blood increases as the blood glucose rises. Similarly, as blood glucose falls, the amount of insulin secreted by the pancreatic islets goes down. In response to insulin, target cells absorb glucose from the blood, having the net effect of lowering the high blood glucose levels to the normal range. In diabetic patients there is an aberration in the functioning of insulin (Guthrie and Guthrie, 1999).

As carbohydrates in food are rapidly metabolized to glucose, the peripheral sugar in blood, insulin is produced by the β -cells in response to rising levels of glucose in the blood after a meal. Insulin makes it possible for body tissues to utilize glucose for fuel or conversion to other molecules or for storage. Insulin also controls the signal for conversion of glucose to glycogen for storage in the liver and muscle cells. Higher insulin levels increase many anabolic processes such as cell growth, cellular protein synthesis and fat storage. Insulin is the peripheral signal in converting many bi-directional processes of metabolism from a catabolic to anabolic direction. If the amount of insulin produced is insufficient, the cells respond poorly to the effects of insulin or if the insulin itself is defective, glucose is not utilized properly by the body cells nor stored appropriately in the liver and muscles. The net effect is persistent high blood glucose levels, poor protein synthesis and other metabolic derangements (Diabetes Control and Complications Trial Research Group, 1993).

Plasma glucose levels principally regulate insulin secretion from β -cells. Increased uptake of glucose by pancreatic β -cells leads to a concomitant increase in metabolism. The increase in metabolism leads to an elevation in the ATP/ADP ratio. This in turn leads to an inhibition of an ATP-sensitive K^+ channel. The net result is a depolarization of the cell leading to Ca^{2+} influx and insulin secretion. In fact, the role of K^+ channels in insulin secretion presents a viable therapeutic agent for treating hyperglycemia due to insulin insufficiency (Pirola *et al.*, 2004; Robinson-White and Stratakis, 2002).

Like the receptors for other protein hormones, the receptor for insulin is embedded in the plasma membrane. The insulin receptor is composed of two alpha subunits and two beta subunits linked by disulfide bonds. The alpha chains are entirely extracellular and house insulin binding domains, while the linked beta chains penetrate through the plasma membrane. The insulin receptor is a tyrosine kinase. In other words, it functions as an enzyme that transfers phosphate groups from ATP to tyrosine residues on intracellular target proteins. Binding of insulin to the alpha subunits causes the beta subunits to autophosphorylate, thus activating the catalytic activity of the receptor. The activated receptor then phosphorylates a number of intracellular proteins, which in turn alters their activity, thereby generating a biological response (Pirola *et al.*, 2004).

In most non-hepatic tissues, insulin increases glucose uptake by increasing the number of plasma membrane glucose transporters known as GLUTs. Glucose transporters are in a continuous state of turnover. Increases in the plasma membrane content of transporters stem from an increase in the rate of recruitment of new

transporters into the plasma membrane, deriving from a special pool of preformed transporters localized in the cytoplasm. GLUT1 is present in most tissues, GLUT2 is found in liver and pancreatic β -cells, GLUT3 is in the brain and GLUT4 is found in heart, adipose tissue and skeletal muscle (Pirola *et al.*, 2004).

All of the post-receptor responses initiated by insulin binding to its receptor are mediated as a consequence of the activation of several signal transduction pathways. These include receptor activation of phosphatidylinositol-3-kinase (PI3K). Activation of PI3K involves a linkage to receptor activation of insulin receptor substrates, which include IRS1, IRS2, IRS3 and IRS4. Activated PI3K phosphorylates membrane phospholipids, the major product being phosphatidylinositol 3, 4, 5 trisphosphate (PIP₃). PIP₃ in turn activates various enzymes, which are thought to initiate many of the metabolic actions of insulin (Shepherd, 2002).

Insulin also has profound effects on the transcription of numerous genes, effects that are primarily mediated by regulated function of sterol-regulated element binding protein (SREBP). These transcriptional effects include increases in glucokinase, pyruvate kinase, lipoprotein lipase (LPL), fatty acid synthase (FAS) and acetylCoA carboxylase (ACC) and decreases in glucose 6-phosphatase, fructose 1,6-bisphosphatase and phosphoenolpyruvate carboxykinase (PEPCK) (Shepherd, 2002).

Epinephrine diminishes insulin secretion by a cAMP-coupled regulatory path. This hormone also counters the effect of insulin in liver and peripheral tissue, where it binds to β -adrenergic receptors, induces adenylate cycles activity, increases cAMP, and activates PKA activity similarly to that of glucagons. In addition, epinephrine influences

glucose homeostasis through interaction with α -adrenergic receptors (Shepherd, 2002). Hormonal control of insulin action is as a result of two gastrointestinal hormones that have significant effects on insulin secretion and glucose regulation. These hormones are the glucagon-like peptides (principally glucagon-like peptide-1, GLP-1) and glucose-dependent insulinotropic peptide (GIP). Both of these gut hormones constitute the class of molecules referred to as the incretins. Incretins are molecules associated with food intake-stimulation of insulin secretion from the pancreas (Robinson-White and Stratakis, 2002).

Insulin is injected into the fat under the skin where it is absorbed directly by the blood. An alternative to injections is an insulin pump (worn outside the body) that delivers insulin through a catheter in the tissue below the skin of the abdomen. The pump eliminates the need for injections and offers better control of blood sugar (Anderson *et al.*, 2001). Insulin is unavailable and unaffordable in many poor countries despite being listed by World Health Organization as an essential drug. Access to insulin by those who require it is a subject of special concern to International Health Agencies and National Health Authorities (WHO, 2003).

1.5.1.2 Oral Hypoglycemic Drugs

Type 2 diabetes is presently treated using five classes of oral hypoglycemic drugs. These drugs are effective in some diabetics but they lose their effectiveness in a significant percentage of patients. Oral hypoglycemic agents lower blood sugar by promoting the pancreas to secrete insulin. These classes of compounds include

biguanides, sulfonylureas, α -glucosidase inhibitors, thiazolidinediones and meglitinides (Jahodar, 1993; Kelly, 1995; Anderson *et al.*, 2001).

1.5.1.2.1 Sulfonylureas

They include drugs such as glipizide, glyburide, and glimepiride. They lower blood sugar by promoting the pancreas to secrete insulin. These are most effective in individuals who still have some working β -cells in the pancreas. The mode of action of sulfonylureas could be chiefly explained by inhibition of *KATP* channels initiating insulin secretion from the pancreatic β -cells (Chakrabarti and Rajagopalan, 2002). Side effects include weight gain. These drugs are also hypoglycemic (Kelly, 1995; Anderson *et al.*, 2001).

1.5.1.2.2 Biguanides

Metformin is a biguanide, which is derived from *Galega officinalis* a medicinal plant (Oubre *et al.*, 1970). The drug acts by reducing plasma glucose through the inhibition of hepatic glucose production and increases glucose uptake in the muscle. It also lowers blood sugar by improving the response to insulin cells in the body. The muscle uses more glucose and the liver makes less glucose. The biguanides are also known to reduce LDL. Weakness, weight loss, nausea, abdominal discomfort, and diarrhea, shortness of breath, dizziness lactic acidosis and kidney toxicity are some of the side effects associated with this class of drugs (Anon, 1998c; Anderson *et al.*, 2001)

1.5.1.2.3 α -glucosidase inhibitors

This includes drugs such as acarbose, precose and miglitol, which are inhibitors of intestinal α -glucosidase (Raing *et al.* 2000). These agents inhibit the breakdown of

complex carbohydrates and delay the absorption of monosaccharide from the gastrointestinal tract (Campbell *et al.* 1996). These two actions decrease postprandial glucose level from the intestines, thereby reducing the rise in blood glucose that occurs after a meal. Side effects include flatulence and interference with the body's absorption of iron, gas accumulation, bloating and diarrhea (Annon, 1998b; Anderson *et al.*, 2001).

1.5.1.2.4 Meglitinides

These drugs include prandin, repaglinide and nateglinide. These drugs are taken with meals and reduce the elevation of blood sugar that follows eating. They enhance insulin secretion from the pancreas. These drugs when taken without meals lower the blood sugar dramatically and inappropriately. Other side effects associated with meglitinides include weight gain and gastrointestinal disturbances (Anderson *et al.* , 2001).

1.5.1.2.5 Thiazolidinediones

These are expensive oral hypoglycemic drugs. Examples include rosiglitazone and pioglitazone. The drugs work to decrease insulin resistance and improve the muscle ability to use insulin. They also decrease plasma triglycerides levels. Molecular mechanisms of action of these agents are through binding to peroxisome proliferator-activated receptor gamma (PPAR γ). PPAR γ is a member of a family of nuclear receptors and is expressed in many tissues, including colon, skeletal muscle, liver, and heart and activated macrophages and is most abundant in adipocytes (Greenfield and Chisholm, 2004).

Thiazolidinediones are selective agonists of PPAR γ . When activated by a ligand, such as a thiazolidinedione, PPAR γ binds to the 9-*cis* retinoic acid receptor (RXR [retinoid X receptor]) to form a heterodimer. This binds to DNA to regulate the genetic transcription and translation of a variety of proteins involved in cellular differentiation and glucose and lipid metabolism (Greenfield and Chisholm, 2004).

Activation of PPAR γ leads to enhanced differentiation and proliferation of preadipocytes into mature fat cells, particularly in non-visceral (peripheral or subcutaneous) fat depots. There is an up regulation of enzymes/transporters in adipocytes like an increase in lipoprotein lipase, fatty-acid transporter 1 and glycerol kinase to facilitate their uptake of fatty acids. Most of these consequences of PPAR γ stimulation are not seen in visceral adipocytes, even though these cells have abundant PPAR γ receptors (Greenfield and Chisholm, 2004). Studies in animals and humans have shown that thiazolidinediones only improve insulin action (and glycaemic control in diabetes) in the presence of insulin resistance. This may be explained by the fact that the effects of these drugs on lipid redistribution are only beneficial if there is excess tissue lipid availability. The 'lipid-steal' effect of thiazolidinediones may therefore be a major contributor to improved insulin action in muscle (enhanced glucose utilization) and liver (reduced hepatic glucose output), as the direct effects of PPAR γ stimulation in muscle and liver are unclear (Greenfield and Chisholm, 2004).

Thiazolidinediones are thought to improve insulin sensitivity by altering hormone production by adipocytes. Adipocytes secrete a number of important hormones, referred to as 'adipokines', including leptin, adiponectin, resistin and tumour necrosis factor- α .

(TNF- α) (Furnsinn and Waldhausl, 2002). The thiazolidinediones, via PPAR γ activation, increase the production of adiponectin, which has been shown to increase fat oxidation, improve insulin action and to have anti-atherogenic properties. They also reduce the secretion of substances which impair insulin action such as TNF- α and, possibly, resistin (Gurnell *et al.*, 2003). Thiazolidinediones are associated with weight gain and increase in LDL-cholesterol, anemia, edema, and liver damage (Annon, 1998a; Anderson *et al.*, 2001).

1.5.1.3 Supplements with Glucose-Lowering Effects

Dietary options are available for people with diabetes mellitus. Supplying diabetic patients with additional key nutrients improves sugar control and helps prevent many major complications of diabetes. Metabolism of several trace elements is altered in insulin-dependent diabetes mellitus and these nutrients have specific roles in the pathogenesis and progress of the disease (Tuvemo and Gebre-Medhin, 1985). Oxyanions, such as vanadate or vanadyl cause insulin-like effects on rats by stimulating the insulin receptor tyrosine kinase. Tungstate and molybdate show the same effects on rat adipocytes and hepatocytes (Matsumoto, 1994).

Since these oxyanions naturally exist in organisms, oxyanion therapy, which is the oral administration of vanadate, vanadyl, molybdate, or tungstate, is considered to be orthomolecular medicine. Therefore, these oxyanions provide an alternative to chemotherapy. Many diseases in addition to diabetes mellitus are also treated since tyrosine kinases are involved in a variety of diseases. Selenium is involved in processes which protect the cell against oxidative damage by peroxides produced from lipid

metabolism. Increased urinary loss of zinc is a commonly encountered feature of diabetes (Tuvemo and Gebre-Medhin, 1985; Matsumoto, 1994; Singh, 1995; Frank *et al.*, 2000). Some of the minerals used are chromium, vanadium, magnesium, zinc and manganese among others (Mertz, 1969; Offenbacher *et al.*, 1980; Sjogren *et al.*, 1988).

1.5.1.3.1 Zinc

Zinc is an essential element in mammals involved in the healing processes, hormone metabolism and immune responses (Walsien, 1976). People with type 1 diabetes (IDDM) are zinc deficient, which may impair immune function. Zinc supplements lower blood sugar levels in people with IDDM. People with type 2 diabetes (NIDDM) have low zinc levels, caused by excess loss of zinc in their urine. High-dose oral zinc enhances wound healing in diabetics (Tuvemo and Gebre-Medhin, 1985). Zinc is essential to the proper functioning of insulin in the body and is necessary for the binding of insulin to the islets of Langerhans in the pancreas, which is necessary for the functional and morphological integrity of β -cells. One of zinc's main uses is regulating the immune system, which is responsible for fighting off harmful viruses and bacteria. Problems with the circulatory and immune systems leads to poor wound healing and dangerous infections in diabetics.

Zinc increases the number and activity of certain types of immune system cells that are especially important for fighting infections. In addition, zinc helps control blood sugar. Since diabetic patients also have low zinc levels, it is important that they get plenty of zinc in their diets. Calf liver, crimini mushrooms and spinach are three very good sources of zinc (Lehman and Spinas, 1996).

1.5.1.3.2 Manganese

Manganese is a trace element that is essential for human health. Manganese (Mn) plays an important role in a number of physiologic processes. It is a constituent of some enzymes and an activator to others (Keen, 2001). Low dietary manganese or low levels of manganese in blood or tissue is associated with several chronic diseases such as osteoporosis, epilepsy and diabetes mellitus (Nicoloft *et al.*, 2004). A number of manganese-activated enzymes play important roles in the metabolism of carbohydrates, amino acids, and cholesterol (Institute of Medicine, 2001).

Pyruvate carboxylase, a manganese-containing enzyme, and phosphoenolpyruvate carboxykinase (PEPCK), a manganese-activated enzyme plays critical roles in gluconeogenesis the production of glucose from non-carbohydrate precursors (Leach and Harris, 1997).

Manganese is a key element in bone development and its deficiency results in abnormal skeletal muscle development in a number of animal species. It is the preferred cofactor of enzymes called glycosyltransferases, which are required for the synthesis of proteoglycans that are needed for the formation of healthy cartilage and bone (Keen and Zidenberg-Cherr, 1996). Manganese metal is used in the making and activation of superoxide dismutase an antioxidant enzyme that help to protect the cell membranes and tissue from degeneration and disruption (Nicoloft *et al.*, 2004). Wound healing is a complex process that requires increased production of collagen. Manganese is required for the activation of prolylase, an enzyme that functions to provide the amino acid, proline, for collagen formation in human skin cells (Keen, 2001).

1.5.1.3.3 Chromium

It is a trace element required for the maintenance of normal glucose metabolism. Chromium is found in a variety of foods and supplements, including liver, brewer's cheese, meats, fish, fruits, vegetables and whole grains (Anderson *et al.*, 2001). Chromium helps insulin pull glucose from the bloodstream into the cells for energy (O'Connell, 2001). Chromium supplements also reduce blood glucose levels in individuals with type 2 diabetes and reduce the need for insulin in those with type 1 diabetes. Joint administration of doses of biotin and chromium picolinate combats insulin resistance, improve β -cell function, enhance postprandial glucose uptake by both liver and skeletal muscle, and inhibit excessive hepatic glucose production (McCarty, 1999).

1.5.1.3.4 Vanadium

Vanadium is an essential trace mineral present in the soil and in many foods. It mimics the action of insulin and in a number of human studies; vanadyl sulfate (a form of vanadium) increases insulin sensitivity in those with type 2 diabetes. Vanadium lowers blood glucose to normal levels (reducing the need for insulin) in diabetics (Tuvemo and Gebre-Medhin, 1983; Matsumoto, 1994)

1.5.1.3.5 Magnesium

Hypomagnesemia is common in patients with diabetes especially those with glycosuria, ketoacidosis, and excess urinary magnesium losses. Hypomagnesemia increases the risk of ischemic heart disease and severe retinopathy (Tuvemo and Gebre-Medhin, 1985). A strong association exists between low levels of magnesium in the blood and type 2 diabetes. Magnesium has no effect on hepatic glucose output and nonoxidative

glucose disposal (O'Connel, 2001; Mooradian *et al.*, 1994). Increased deficiency of magnesium potentially causes states of insulin resistance. Magnesium has a role in the evolution of such complications as neuropathy, retinopathy, thrombosis, and hypertension. Low magnesium levels worsen blood sugar control and that food rich in magnesium (such as whole grains, green leafy vegetables, bananas; legumes, nuts, and seeds or magnesium supplements) promote healthy blood glucose levels. Taking magnesium supplements improves the action of insulin and decrease blood sugar levels, particularly in the elderly (Anderson *et al.*, 2001).

1.5.1.3.6 Potassium

Potassium supplementation yields improved insulin sensitivity, responsiveness and secretion in diabetics; insulin administration induces a loss of potassium; and a high potassium intake reduces the risk of heart disease, atherosclerosis, and cancer (Norbiato *et al.*, 1984; Khaw and Barrett-Connor, 1984).

1.5.1.3.7 Inositol

Inositol is needed for normal nerve function. Diabetes causes nerve damage, or diabetic neuropathy. Some of these abnormalities are reversed by inositol supplementation (Gegersen *et al.*, 1999).

1.5.1.3.8 α -lipoic acid and γ -linolenic acid

α -lipoic acid (ALA) is a powerful natural antioxidant and is used to improve diabetic neuropathies and reduces pain. γ -linolenic acid (GLA) found in black currant seed oil, borage oil, and evening primrose oil, is also helpful in improving damaged nerve function common in diabetes (Jacob *et al.*, 1999).

1.5.1.3.9 Carnitine

Carnitine is a substance needed by the body for proper utilization of fat for energy. When diabetics are given carnitine at 1 mg per kg of body weight, high blood levels of fats is reduced by 25-39% in ten days. In addition, carnitine improves the breakdown of fatty acids, and therefore prevents diabetic ketoacidosis (Giancaterini *et al.*, 2000).

1.5.1.3.10 Fiber

A high-fiber diet helps prevent development of type 2 diabetes. High fiber diet also improves cholesterol and triglyceride levels in those with diabetes (Anderson *et al.*, 2001). High fiber diets are uniformly recommended for diabetics. Insoluble fiber is more characteristic of brans and husks of whole grains, nuts, and seeds. Soluble fibers include pectins, fruits, vegetables gums, and mucilages, which act to increase the viscosity of food in the intestine, thus slowing or reducing the absorption of glucose (Broadhurst, 1997)

1.5.1.3.11 Antioxidants

Antioxidants such as β -carotene, vitamin E and vitamin C are scavengers of free radicals, which are unstable, and potentially damaging molecules generated by normal chemical reactions in the body. Because insulin resistance is associated with cardiovascular disease, people with diabetes benefit from nutrients that help manage elevated blood lipid levels, high blood pressure, or congestive heart failure. In addition, to lowering blood glucose, antioxidants improve cholesterol levels in people with type 2 diabetes. Elevated levels of free radicals cause damage to nerves and blood vessels

leading to neuropathy. Antioxidant supplements improve nerve communication in damaged areas and reduce the symptoms of diabetic neuropathy (Charalambous, 1994; Duke, 1997).

1.5.1.3.12 Biotin

This is a vitamin B-complex that is helpful for both type 1 and type 2 diabetes and is found in brewer's yeast. Administration of high-dose biotin improves glycemic control. Biotin can substantially lower fasting glucose in type 2 diabetics, without side-effects (Mc Carty, 1999).

1.5.1.4 Exercise

Exercise plays an important role in controlling diabetes because it lowers blood sugar and helps insulin to work more efficiently in the body. Exercise also enhances cardiovascular fitness by improving blood flow and increasing the heart's pumping power. It also promotes weight loss and lowers blood pressure.

Exercise has value, only when it is done regularly at least three to four sessions per week for 30 to 60 minutes per session. People with type 2 diabetes who exercise regularly lose weight and gain better control over their blood pressure, thereby reducing the risk for cardiovascular disease (a major complication of diabetes). People with type 1 diabetes who regularly exercise reduce their need for insulin injections. Despite the benefits of exercise, however, many people have difficulties in sticking with an exercise program for a long period of time.

Healthcare practitioners can help develop suitable routines as well as strategies that may improve adherence to such routines. Anyone with long-standing diabetes should

undergo a thorough screening before beginning an exercise program and should be monitored carefully by his or her physician. Exercise therapy is usually individualized to account for the patient's interest, motivation and physical status (American Diabetes Association, 1995). Exercise should be started at a low level and gradually increased to avoid adverse effects such as injury, hypoglycemia or cardiac problems (Alexandria, 1994; Anderson *et al.*, 2001)

1.5.1.5 Acupuncture

Acupuncture is a physical intervention therapy that is used in diabetes mellitus management. This practice is widely practiced as an alternative therapy for chronic pain. Therefore, relatively few people have turned to acupuncture for treating diabetes. Increasingly, people with pain and other health problems for which acupuncture is selected also have diabetes (Hui, 1995; Dharmananda, 2003). Acupuncture may trigger the release of natural painkillers and reduce the debilitating symptoms of neuropathy (nerve damage) a complication of diabetes. In one study of diabetics suffering from chronic painful neuropathy, acupuncture reduced pain and improved sleep in 77% of the participants and eliminated the need for pain medications in 32% of the participants. Acupuncture may therefore be a reasonable option for diabetics with neuropathy who either find no symptom relief or develop side effects from conventional drug treatment.

The therapy has been observed experimentally and clinically and experiments show that acupuncture can activate glucose -6- phosphatase and affect the hypothalamus. The practice can also act on the pancreas to enhance insulin synthesis, increase the

number of receptors on target cell and accelerate the utilization of glucose, resulting in lowering of blood sugar (Chen and Wei, 1985; Hui, 1995; Huang, 1996).

1.5.1.6 Herbal Management of Diabetes Mellitus

From the earliest times medicinal plants have been crucial in sustaining the health and well being of mankind. They form an indispensable source of both preventive and curative medicinal preparations (Dery *et al.*, 1999; Chevalier, 2001; Musila *et al.*, 2003). Ayurvedic literature shows that since the time of Charak and Sushrut, many herbal medicines in different formulations have been used to manage diabetes mellitus (Chattopadhyay *et al.*, 1993).

Many traditional cultures practice customs that involve traditional or folk remedies. The cultures that are commonly known include the Ayurveda, which is a healing practice rooted in India. Japanese, Chinese, Korean and Southeast Asian traditions involve herbal powders and teas. Other practices which involved uses of folk remedies involving herbs and heavy metals include Hispanic and Caribbean healing practices. The ancient Mayan, Aztec and Inca civilizations had a profound understanding of local medicinal plants (Keen *et al.*, 1994; Nuttfall and Flores, 1997; Borins, 1998; Chevallier, 2001).

Modern oral hypoglycemic drugs were originally derived from herbs. Other drugs such as digitalis; narcotics, atropine and senna have their origins from plants (Vann, 1998). Traditional medicine is used by a large proportion of the population in many countries. The World Health Organization (WHO) estimates that up to 80% of the world's population, mostly in developing countries, relies on traditional medicine

practices for its health care needs. This is particularly true for the poorer sections of the population in developing countries because natural remedies are not only cheaper than modern medicines, but are often the only medicines available in remote rural regions (Geoffrey, 1996; GTZ, 2001).

In Africa, in particular, traditional medicine has always existed and has been practiced since time immemorial. Besides serving medical and cultural functions, medicinal plants in Africa and other developing countries frequently provide economically disadvantaged groups such as small holders and landless people with their only form of cash income (Hirt and Pia, 1995; GTZ, 2001).

In Kenya, traditional medicine continues to play a major role in Primary Health Care (PHC). More than 70% of the Kenyan population relies on traditional medicine as its primary source of health care (Odera, 1997). It is more accessible than modern health facilities for most of the population in the country. It is relatively inexpensive, locally available, and usually accepted by the local communities compared to modern conventional medicine. The substantial contribution to human health and well being of medicinal plant species is now widely appreciated and understood. Indeed, there is a growing demand for many of the species and an increasing interest in their use. Medicinal plants have the advantage of having little or no side effects. The many side effects associated with conventional treatment of diabetes with oral hypoglycemic drugs and insulin, the inadequacy of other forms of therapies such as diet, exercise, mineral supplement and physical interventions such as acupuncture have necessitated patients suffering from diabetes mellitus to seek more reliable and comfortable form of therapy,

and herbal medicine is the most commonly used alternative medicine therapy (Eisenberg, 1997; Brody, 1998).

Medicinal plants are also important sources of therapeutic agents in the industrial production of pharmaceuticals (Lambert *et al.*, 1997). Many of these plants were used for many centuries and some times as regular constituents of the diet; it is assumed that they do not have many side effects. However, chronic consumption of large amounts of traditional remedies must always be taken with caution, as toxicity studies have not been conducted for most of these plants (Shnkar *et al.*, 1980).

The technical inadequacies of modern medicines and the concern over the side effects which has made many feel that the medicine is unnatural coupled with their rising cost have made many patients turn to more gentle alternative herbal medicines (Kokwaro, 1993; Vann, 1998; Angell and Kassirer, 1998; Dey *et al.*, 2002).

Medicinal herbs used in the management of diabetes are known to contain natural compounds with antidiabetic activity. These compounds include complex carbohydrates, alkaloids, glycopeptides, terpenoids peptides and amines steroids, flavonoids, sulfur lipids, coumarins, inorganic compounds and ions. These natural compounds are said to play an important role in maintaining the normal blood glucose level (WHO, 1985; Marles and Farnsworth, 1994). Crude extracts from plants such as *Allium cepa*, *Allium sativum*, *Azadirachta indica*, *Ocimum sanctum* and *Vinca rosea* have been shown to have hypoglycemic activity (Chattopadhyay *et al.*, 1993).

Trigonella foenum graecum (fenugreek) is another Ayurvedic traditional plant with proven activity. In addition to mucilage, fenugreek also contains protein, saponins,

and the hypoglycemic phytochemicals coumarin, fenugreekine, nicotinic acid, phytic acid, scopoletin and trigonelline (Marles and Farnsworth, 1994; Duke, 1997)

Onions and garlic have been used in many cultures to treat diabetes. Garlic, a member of the lily family is most commonly used for flavorful cooking worldwide (Shane, 2001; Sheela and Augusti, 1992). Onions and garlic have blood sugar lowering effects (Sharma *et al.*, 1977; Sheela and Augusti, 1992). The volatile oils in raw onion and garlic have been shown to lower fasting glucose concentration in both diabetic animals and human subjects (Jain *et al.*, 1973).

The active components are allylpropyl disulfide (APDS) and diallyl disulfide oxide respectively. The mechanism of action is thought to be by competing with insulin for insulin non-activating sites in the liver, which results in an increase of free insulin (Sheela and Augusti, 1992).

Ocimum sanctum (holy basil) is another commonly used herb in Ayurveda (related species include *Ocimum album* and *Ocimum basilicum*). Studies in animal models have demonstrated that it has hypoglycemic activity (Chattopadhyay, 1993). Holy basil has effects that include enhanced β -cell function and insulin secretion. Clinical trial of *Ocimum sanctum* showed positive effects on both fasting and postprandial glucose in patients with type 2 diabetes using a local preparation of fresh leaf powder mixed in water for 4 weeks (Agrawal *et al.*, 1996).

Ficus carica (fig leaf) is a popular plant used for patients with diabetes in Spain and other areas in Southwestern Europe. Its active component is unknown. Several studies in animal models with diabetes have shown it to have both the short and long-

term hypoglycemic effects. Potential hypolipidemic effects in diabetic rats have also been demonstrated (Campillo *et al.*, 1991; Torres *et al.*, 1993; Perez *et al.*, 1996). Its effects on glucose are unknown; however, some studies suggest that it facilitates the uptake of glucose peripherally (Serraclara *et al.*, 1998).

Opuntia streptacantha (nopal) or the prickly pear cactus is found in arid regions throughout the Western hemisphere. It has a high soluble, fiber and pectin content, which may affect intestinal glucose uptake, partially accounting for its hypoglycemic actions (Shapiro and Gong, 2002). Animal model studies have reported decreases in postprandial glucose and HbA1c with synergistic effects with insulin (Fрати *et al.*, 1991).

Gymnema sylvestre is another commonly used herb in Ayurveda. The plant is a woody climber that grows in tropical forests of central and southern India. According to common folklore, chewing the leaves causes a loss of sweet taste. Studies of an ethanol leaf extract, GS4, in diabetic rat and rabbit models have reported regeneration of islets of Langerhans, decreases in blood glucose, and increases of serum insulin (Shanmugasundaram *et al.*, 1990). Mechanism of action is unknown although it is postulated that it causes an increase in glucose uptake and utilization, increases insulin release through increased cell permeability, increase in β -cell number, and stimulation of β -cell function (Persand *et al.*, 1999; Shane, 2001).

Ackee fruit (*Blighia sapida*) is a tropical fruit belonging to the Sapindaceae family. It has its origin in West Africa but has traversed the Atlantic to the Caribbean. The trivial name ackee, is derived from the terms “anke” and “akye-fufuo” which are used to describe the fruit in West Africa. Consumption of the ackee is mainly in Jamaica, Haiti

and some parts of West Africa. In Jamaica, the fruit serves as a major component of the national dish ackee and codfish. The ackee has been the cause of widespread epidemics both in Jamaica and in West Africa. From as early as the 19th century there were speculations that the fruit may be toxic. It was not until 1955, however, that the actual causative factor of its toxicity was elucidated. A non-proteinogenic amino acid, hypoglycin A, so named due to its ability to induce severe hypoglycemia (Goldson, 1992). Its chemical structure was elucidated in 1958 and scientifically it is referred to as L-alpha-amino-beta-methylene cyclopropane propionic acid. Hypoglycin A is found predominantly in the immature fruit. Concentrations within the arilli ranges from over 1000 ppm in the immature fruit to less than 0.1 ppm in the fully mature fruit. Ill effects occur only when the immature fruit is consumed. Hypoglycin A is transaminated to methylenecyclopropyl-alanine (MCPA) and subsequently undergoes oxidative decarboxylation to form MCPA-CoA. MCPA-CoA exerts its effect by inhibiting several coenzyme A dehydrogenases, which are essential for gluconeogenesis. Depletion of glucose reserves and the inability of cells to regenerate glucose lead to hypoglycemia (Goldson, 1992).

Momordica charantia is a vegetable indigenous to tropical areas, including India, Asia, South America, and Africa. It is also known as balsam pear, karela (karolla), and bitter melon. Common preparations of the herb range from injectable extracts to fruit juice and fried melon bits (Leatherdale *et al.*, 1981; Welhinda *et al.*, 1986; Srivastava *et al.*, 1993; Shane, 2001). Its active components include charantin, vicine, and polypeptide-p, an unidentified insulin-like protein similar to bovine insulin. The

mechanism of action includes increased insulin secretion, tissue glucose uptake, liver and muscle glycogen synthesis, glucose oxidation, and decreased hepatic gluconeogenesis. Studies in alloxan-induced diabetic rabbits demonstrated hypoglycemic activity (Akhatar *et al.*, 1981).

Aloe vera is the most well known species of Aloe, a desert plant resembling the cactus in the Liliaceae family. It is popularly used to treat burns and promote wound healing. The dried sap of the *Aloe vera* is a traditional remedy for diabetes in the Arabian Peninsula (Pandey *et al.*, 1995).

Pterocarpus marsupium is used in India as a treatment for diabetes. The flavonoid epicatechin extracted from the bark of this plant, has been shown to prevent β -cell damage in rats. Further, both epicatechin and a crude alcohol extract of *Pterocarpus marsupium* have been shown to regenerate functional pancreatic β -cells in diabetic animals (Chakravarthy *et al.*, 1981; Chakravarthy *et al.*, 1982).

Vaccinium myrtillus (bilberry) is a perennial plant that grows in the woods and forest meadows. Bilberry leaf tea has a long history of folk use in the treatment of diabetes. Oral administration of Bilberry leaf tea reduces hyperglycemia in normal and diabetic dogs, even when glucose is concurrently injected intravenously (Bever and Zahnd, 1979). The anthocyanoside myrtillin, extracted with a slightly acidic ethanol solution, is the most active constituent. It is weaker in activity than insulin but it is less toxic, even at 50 times the therapeutic dose. A single dose produces a beneficial effect lasting for several weeks.

Vaccinium myrtillus anthocyanosides provide other benefits in diabetics. Specifically, they have been shown to increase intracellular vitamin C levels, decrease the leakiness and breakage of small blood vessels, prevent easy bruising, and exert potent antioxidant effects. These effects are important in dealing with the microvascular abnormalities of diabetes (Passariello *et al.*, 1979; Caselli 1985).

Medicinal herbs used in indigenous medicines for the management of diabetes mellitus contain both organic and inorganic constituents. Some of these inorganic trace elements possess antidiabetic properties, which accounts for the activity of medicinal herbs.

Evaluation of a traditional medicine is carried out by identification, which is done by acquiring ethnomedical clinical data, which include pharmaceutical information. Ethnobotanical data, which includes botanical, ecological and pharmacognostical information, is also needed. Chemical data that includes phytochemistry and chemotaxonomy too is required. Information from the practicing herbalist is also an important aspect in evaluating traditional medicine. There is need to further investigate these plants so as to ensure their safety and efficacy. Laboratory animal experimentation are used to determine the safety of the medicine to a high degree of accuracy and confirm the information gathered from the traditional medicinal practitioner.

The seven plants assessed for blood glucose lowering effect and their safety in this study are:

Caesalpinia volkensii (Caesalpinaceae) is known locally as Mubuthi in Mbeere. It is a woody climber with recurved sharp prickles. The plant has pinnate leaves, yellow

flowers and flat pods. It is used to treat many diseases, the most common being malaria. Pounded leaves are used in soups and tea mainly for malaria. Roots are used for gonorrhoea and bilharzias while seeds are used for stomach ulcers. Buds are crushed for eye troubles and the Shambaa and Bondeni communities use it as an aphrodisiac (Gacathi, 1989; Kokwaro, 1993). Aqueous decoctions, ethanol macerates, and petroleum ether, methanol and water Soxhlet extracts of *Caesalpinia volkensii* are active against malaria (Kuria *et al.*, 2001).

Shrub of *Vernonia lasiopus* (Compositae), which grows in the wild reaches up to 2 metres. The local name for *Vernonia lasiopus* is Mucatha in Mbeere. The plant has toothed leaves and purple flower heads. A decoction from the leaves and roots is used in the treatment of intestinal worms in sheep (Gacathi, 1989). Its leaves are chewed or boiled to cure stomach ache (Kokwaro, 1993). Methanol extracts of *Vernonia lasiopus* are active against *P.falciparum* singly and in combination with chloroquine (Muregi *et al.*, 2003).

Carissa edulis (Apocynaceae) belongs to the family Apocynaceae and its vernacular name is Mukawa in Mbeere. This is a common plant of dry areas (Gacathi, 1989; Beentje, 1994). *Carissa edulis* is an evergreen shrub common in most districts in Kenya. The shrub is branched spiny and grows to a height of up to 4 m high. Flowers are sweetly scented; pinkish white fruits. They are edible and dark purple when ripe. The bark produces milky latex on cutting. *Carissa edulis* is used traditionally for the treatment of headache, chest complaints, rheumatism, gonorrhoea, syphilis, and rabies and as a diuretic (Nedi *et al.*, 2004). Root decoction is also used for malaria treatment and also for

indigestion and lower abdominal pains when one is pregnant (Kokwaro, 1993). Oral administration of the ethanolic extracts of *Carissa edulis* leaves on streptozotocin (STZ) induced diabetic rats reduced the blood glucose level in STZ diabetic rats during the first three hours of treatment (El-Fiky *et al.*, 1996).

Ficus sycomorosa (Moraceae) trees grow up to about 25m in height. Its vernacular name is Mukuyu in Mbeere. Figs which are red when ripe are produced from the trunk. The milky juice is used to relieve toothache. The bark is used in the treatment of liver troubles and diarrhea, while bark decoction is taken for the treatment of general abdominal pains and stomach disorder (Kokwaro, 1993; Gacathi, 1989).

Kleinia squarrosa (Asteraceae) grows in Mbeere and Machakos districts of Eastern province in Kenya. The vernacular name is Mungendia Nthe'nge in Mbeere. The plant is used locally in the treatment of asthma, amenorrhea, syphilis, jaundice, and malaria. The stem is usually boiled or pounded and soaked and the mixture taken as a decoction or infusion (Musila *et al.*, 2003).

Azadirachta indica (Meliaceae) whose common name is neem has been reputed to possess cardiovascular, antimicrobial, immunomodulatory, hypoglycemic and a number other effects. A bitter principle, nimbi din, isolated from seeds of neem tree is effective in reducing fasting blood glucose at a dose of 200mg/ kg in alloxan diabetic rabbits. Aqueous extract of tender neem leaves have been reported to reduce blood glucose and this effect is due to blocking the action of epinephrine on glycogenolysis and peripheral utilization of glucose. Studies have also shown that neem has moderate

reversal of diabetic retinopathy in streptozotocin induced diabetic rats (Eshrat and Hussain, 2002; Khosla *et al.*, 2000).

Helichrysum odoratissimum (Compositae) belongs to the family compositae. The local name is Mutaa in Mbeere. The plant is a weak shrub and is common along the forest edges and clearings (Gacathi, 1989). Leaves of this plant are used as anthelmintic; pounded leaves are applied over wounds resulting from burns. Young branches are used as eye drops for conjunctivitis while roots are crushed and steeped in hot or cold water and the extract used as a purgative (Kokwaro, 1993).

1.6 Justification

Diabetes mellitus is a predominant public health concern. WHO projects a 170 % growth in the number of people with diabetes in the developing countries by 2025. This is equivalent to 84 to 228 million people (WHO, 1998). It causes substantial morbidity, mortality, and long-term complications and remains an important risk factor for cardiovascular disease. With increasing rates of childhood and adult obesity, diabetes is likely to become even more prevalent over the coming decade. The combination of rising prevalence of diabetes and the high rate of long term complication especially in Africa will lead to drastic increase of the burden of diabetes on the health systems of African counties most of which face economic difficulties. Possible prevention strategies have been hindered by the scarcity of data on diabetes in Africa. Many of the conventional drugs that are available for the management of the disease are not only expensive but also have numerous side effects, which lead to complications. Herbal medications on the other hand are cheaper and locally available. Many of the plant

species growing throughout the world contain active constituents that offer benefits that conventional drugs lack, helping combat illness while supporting the body's efforts to regain good health.

Many plants have been traditionally used to manage diabetes without authentication on their antidiabetic properties and assessment of their safety. The use of crude extracts without pharmacological and toxicological evaluation could lead to serious complications and even death. Since many of the conventional drugs have originated from medicinal herbs, a study of these plants may lead to the discovery of drugs with higher efficacy and low toxicity.

1.7 Hypothesis

The aqueous extracts from medicinal plants as used by traditional medicine practitioners (TMP) have hypoglycemic properties.

1.8 Main Objective

To evaluate seven Kenyan medicinal plants for their potential to manage diabetes mellitus and their safety.

1.8.1 Specific Objectives

- i. To screen aqueous extracts from some plants used in traditional medicine for *in vivo* hypoglycemic activity.
- ii. To determine the trace element composition and content in water extracts from the plants used in traditional medicine to manage diabetes mellitus.
- iii. To determine the classes of compounds present in the plants used in traditional medicine to manage diabetes mellitus

- iv. To evaluate preliminary toxicity of the water extracts from the seven plants used in traditional medicine to manage diabetes mellitus through observation of histological sections.

Seven medicinal plants were collected from the forest of the University of Nairobi during the rainy season. Leaves, stems, roots, bark, and seeds of the following botanical families were identified: *Simarubaceae*, *Umbelliferae*, *Leguminosae*, *Convolvulaceae*, *Scrophulariaceae*, *Ericaceae*, and *Myrsinaceae*. The seven plants investigated for antidiabetic activity were *Albizia adansonioides*, *Albizia leucodermis*, *Albizia saman*, *Albizia zizanioides*, *Albizia zizanioides*, *Albizia zizanioides*, and *Albizia zizanioides*.

The various parts of the plants collected were dried in the shade for 72 hours. The shade-dried stems, leaves, and roots were harvested and cut into small pieces and dried at 25°C for 72 hours. The dried materials were then crushed into powder by use of an electric mill (Nairobi and Nairobi Ltd, Kenya). The powder was then immediately used for extraction of the active ingredients. The powder was then stored in airtight containers.

2.1 Preparation of Extracts

Plant extracts were prepared by 100% methanol extraction of the dried plant material for two hours with constant stirring. The mixture was left to cool at room temperature and then filtered through a dry filter paper into a clean 250 ml Erlenmeyer flask. The second extraction was then filtered using the same procedure as the first extraction. The filtrate was then dried to 100% methanol by a rotary evaporator.

CHAPTER TWO

MATERIALS AND METHODS

2.1 Collection of Plant Materials

Seven medicinal plants were collected from their natural habitat in various parts of Mbeere district in Eastern province, Kenya based on the folklore reports from practicing herbalists on their anti-hyperglycemic activity. An acknowledged authority identified the plants and voucher specimens were deposited at the National Museum of Kenya herbarium. The seven plants investigated for antidiabetic activity included *Helichrysum odoratissimum*, *Azadirachta indica*, *Ficus sycomorus*, *Carissa edulis*, *Vernonia lasiopus*, *Caesalpinia volkensii* and *Kleinia squarrosa*.

The various parts of the plants collected were root barks, leave and stem barks. The stem barks, root barks and roots were harvested and cut into small pieces and air-dried at $25\pm 3^{\circ}\text{C}$ for one month. The dried materials were then crushed into powder by use of an electric mill (Christy and Norris Ltd England). The material that was not immediately used was stored in plastic bags and kept at room temperature away from direct sunlight.

2.2 Preparation of Extracts

Plant extracts were prepared by boiling 100 g of crushed material in one liter of distilled water for two hours with frequent stirring. The mixture was left to cool at room temperature and then decanted into a dry clean conical flask through folded cotton gauze stuffed into a funnel. The decanted extract was then filtered using filter papers by use of a vacuum pump. The filtrate was freeze-dried in 150 ml portion using a freeze drier for 72

hours. Afterwards the powder was pooled together and stored at 4°C in airtight containers.

2.2.1 Preparation of Extracts for Injection into Mice

Physiological saline was prepared by dissolving 0.85g of analytical grade sodium chloride in 100 ml of distilled water. The extracts for injection were prepared as follows: the 50mg/kg body weight dose was prepared by dissolving 12.5mg in 1ml of physiological saline; the 100mg/kg body weight dose was prepared by dissolving 25mg in 1ml of physiological saline; and the 150mg/kg body weight dose was prepared by dissolving 37.5mg in 1ml of physiological saline. The animals were given 0.1 ml of the extract solutions. Insulin was also reconstituted and animals in group 2 were given 0.1 ml. The animals in groups 1 and 3 were also injected with 0.1 ml physiological saline intraperitoneally.

2.3 Pharmacological Testing

2.3.1 Animals

Health Swiss albino male mice were used for the study. The animals were 4-6 weeks old and weighed 23-27 g, and were fed on the standard mice diet and allowed free access to water *ad libitum*. For experimental purposes, the animals were fasted overnight and allowed free access to water. The animals were divided into 4 groups of four animals each. The animals in group 2, 3 and 4 were alloxanized using alloxan 4-6 days before the start of the experiment. Group 1 was not alloxanized and were given 0.1ml of normal saline; Group two was given 0.1 ml of normal saline; the third group of animals were given insulin at the dose of 1 unit per kg body weight; group 4 animals was treated with

plant extracts at three dose levels: 50mg/kg body weight, 100mg/kg body weight and 150 mg/kg body weight. Each dose level consisted of 4 animals. Group 1 and 2 served as the experimental controls while group 3 served as the reference. Alloxan is a chemical that kills the pancreatic islets cells that are responsible for insulin production. Once the β -cells are destroyed, the pancreas cannot produce insulin and the animal becomes diabetic. The alloxan monohydrate 10 g used in this experiment was obtained from Fluka chemie GmbH ch 9471 Switzerland and injected intraperitoneally to mice at a dose of 150mg/kg body weight. Insulin was given to mice at a dose of one unit per kg/body weight (1IU/kgbw) intraperitoneally.

Before administration of the different treatments the animals were bled and blood glucose level in the animals was measured. This was the initial measurement at time zero hour. The animals were again bled hourly until the fourth hour.

2.3.2 Collection of Blood Samples

Blood samples were collected from the tails of the animals after wiping the tail with surgical spirit. The tail was nibbled by use of a pair of sharp scissors; a drop of blood was squeezed into a Supreme hypoguard meter. After collection of blood, the nibbled side of the tail was rubbed with cotton wool soaked in absolute alcohol to protect the animal from infection and to arrest bleeding.

2.3.3 Blood Glucose Determination

The principle of the test is based on a glucose oxidase/peroxidase reaction, which is specific for β -D-glucose. The hypoguard machine was used together with GB Supreme blood glucose test strips. The Supreme Test Strip is a disposable plastic strip containing a

chemically treated test area used to measure the amount of blood glucose. The test area is designed in such a way that when a drop of blood is placed on the top surface, color change occurs which is determined by a Supreme hypoguard meter. The supreme Test Strip was fully inserted into the meter before applying a drop of blood to fully cover the test area inside the grey target. The supreme test strips and the supreme hypoguard meter were obtained from hypoguard Ltd, United Kingdom through Chemoquip Ltd, Kenya.

2.4 Phytochemical Screening

Phytochemical screening was undertaken to determine the class of compounds present in the plants extracts using the method described by Houghton and Raman (1998). Plants extracts were screened for the presence of alkaloids, saponins, tannins, terpenoids, sterols, flavonoids and anthraquinones.

2.4.1 Alkaloids

0.5 g of each plant extract was stirred in 2 ml of 1% aqueous HCl and heated in a boiling water bath for 10 minutes. The mixture was then filtered while hot and treated with Dragendorff's reagent. Orange precipitate indicates the presence of alkaloids.

2.4.2 Sterols and Terpenoids

0.5g of the extract was stirred with hexane to defat it. The residue was extracted with 1 ml dichloromethane. The dichromethane solution was dried over anhydrous sodium sulphate. 1 ml portion of the dichloromethane was mixed with 0.5 ml acetic anhydride followed by two drops of sulphuric acid. A gradual appearance of green to blue colors is taken as indicative of sterols. A color change from pink to purple indicates the presence of terpenoids.

2.4.3 Saponins

0.5 g of the plant extract was shaken with 2 ml water in a test tube. Frothing which persisted for at least half an hour was a positive test for saponins (Rukunga, 1984).

2.4.4 Flavonoids

0.5 g of the extract was defatted by several washing in hexane. The defatted residue was washed in 4 ml 80% methanol and filtered. The filtrate was treated as follows: To one 2 ml portion of the filtrate, 1 ml of 1% aluminum chloride in methanol was added. Development of yellow color was indicative of the presence of flavonols, flavones and chalcones. To another 2 ml portion of the filtrate, 1 ml of 1% potassium hydroxide was added. Development of a dark yellow color was indicative of the presence of flavonoids.

2.4.5 Tannins

1 g of extract was stirred in 2 ml of distilled water and then filtered. Ferric chloride was added into 2 ml of the filtrate. The formation of a blue black to green precipitate was evidence of the presence of tannins.

2.4.6 Anthraquinone

In one sample, 0.5 g of the plant extract was shaken with 2 ml of benzene and filtered. A 10% ammonium hydroxide solution (1 ml) was added and the mixture shaken. A red color if observed in the ammoniac phase was indicative of the presence of free anthraquinones.

In another sample, 0.5 g of a benzene washed plant extract was boiled with 2 ml of 1 % HCl and filtered while hot. The filtrate was then shaken with 1 ml benzene. The

benzene layer was removed and 10% ammonia hydroxide added. A red color if observed was indicative of the presence of bound anthraquinones.

2.5 Trace Elements Determination

2.5.1 Energy Dispersive X-ray Fluorescence (EDXRF)

The different trace elements present in the extracts were investigated by use of Energy Dispersive X-ray fluorescence analytical technique. 0.3g of the freeze-dried material was weighed and made into pellets of 2.5 cm in diameter and weighing 100-200 mg/cm². The pellets were made using a pellet press machine. The pellets were weighed and their weights recorded. The EDXRF system consists of an X-Ray spectrometer and a radioisotope excitation source. The radiation from the radioactive source, Cd¹⁰⁹ (T_{1/2} = 453 days and activity = 10 mCi) are incident on the sample, which emits the characteristic X-Rays. These X-Rays are detected by Si (Li) detector (EG&G Ortec, 30mm²x10mm sensitive volume, 25µm Be window) with energy resolution of 200eV at 5.9keV Mn K α – line. The spectral data analysis was collected using personsl computer based Cariberra S-100 multichannel analyser (MCA). The acquisition time applied in the EDXRF measurements was 1000seconds. The X-Ray spectrum analysis and quantification was done using IAEA QXAS software (QXAS, 1992), which is based on on the Fundamental Parameters Method (FPM) (Sparks, 1975;Giauque *et al.*, 1973,1977).

2.5.2 Atomic Absorption Spectrophotometry (AAS)

This technique was used for analysis of magnesium, chromium and vanadium. The machine used was Buck model 210 VGP Atomic absorption spectrophotometer. The VGP AAS is designed to measure the concentration of elemental metals in solution. It

provides integrated measurements in absorbance or emission intensity as well as sample concentration in comparison to standard solutions. Readings can be integrated over a period from 0.5 seconds to 10 seconds.

2.5.2.1 Preparation of Standard Solutions

Standard stock solutions of 1000 parts per million (ppm) for AAS were used as supplied by the manufacturers (Aldrich Chemical Co. Inc).

2.5.2.2 Preparation of Working Standards

Suitable aliquots of standard stock solutions of each element were taken in a series of 100ml volumetric flasks. The solutions were diluted to volume using distilled-deionised water, mixed thoroughly and transferred into plastic beakers. This was done for each element when its analysis was to be undertaken. For each element, working standard solutions were prepared within a given range (1 ppm, 5 ppm, 10 ppm, 15 ppm, 20 ppm, and 25 ppm). The relationship between concentration and absorbance was linear. In case of magnesium, 2 ml of 5% Lanthanum solution was added to each series of the working standard solution before diluting them to volume. Standard blank reagents for each element were prepared by adding all the used reagents, except the target element being determined.

2.5.2.3 20% Hydrochloric Acid (w/w)

The solution was prepared by transferring 548 ml of concentrated hydrochloric acid (36%) of analar grade carefully into 300ml of distilled- deionised water contained in 1 litre volumetric flask. The solution was then diluted to one litre, mixed thoroughly and kept in plastic bottles.

2.5.2.4 Lanthanum Solution

Lanthanum solution (50mg/ml) was prepared by dissolving 12.6263g of Lanthanum chloride in distilled-deionised water. The solution was then diluted to 250ml with distilled-deionised water in a volumetric flask. After mixing thoroughly, the solution was kept in clean plastic bottle and used during the determination of magnesium in the plant materials.

2.5.3 Digestion of Plant Materials

Each plant material that was collected for the study was brought to solution by wet oxidation/digestion. The procedure was repeated two times. Wet oxidation for determination of magnesium (Mg), vanadium (V), and chromium (Cr) was done as follows:

The dried samples of known weights were transferred into 100 ml Pyrex beakers and to each beaker, 10 ml of concentrated Nitric acid (HNO_3) was added, then allowed to soak thoroughly. 3 ml of 60% Perchloric acid (HClO_4) was added to each beaker, then warmed on hot plate slowly at first, until frothing ceased. Heating was then intensified until all Nitric acid was evaporated. When charring occurred, the mixture was cooled, 10 ml of Nitric acid was added and heating continued until white fumes of Perchloric acid were observed. The final solution was cooled and 25 ml of 20% Hydrochloric acid (HCl) was added. The solution was then transferred into 100 ml volumetric flask by filtering through Whatman filter paper No.1. The solutions were then made to volume and shaken well to allow proper mixing before the contents were transferred to plastic sample bottles. The samples were kept in a freezer awaiting analysis.

2.5.4 Total Elemental Content Determination

Wet digests of the plant materials were analyzed for Mg, V and Cr. Working standards were prepared according to the procedure given in section 2.5.2.2. Sample solutions for analysis of magnesium were prepared by withdrawing 1 ml of the digested sample solution into 100 ml volumetric flasks. 5% Lanthanum solution was added in each flask and the mixture diluted to volume using distilled-deionised water. However, for analysis of vanadium and chromium, the digested sample solutions were analyzed without dilution.

After setting the AAS instrument to the right conditions for each element, the respective standard and sample solutions were aspirated into the flame in turns to determine their respective absorbance. At least four standard solutions were aspirated between 6-10 samples to monitor the stability of the working conditions. Distilled-deionised water was always flushed into the flame to re-establish the zero absorbance. For each sample and element, the above procedure was repeated two times. The mean absorbance for each sample solution and standard solutions were calculated and recorded.

To prepare a calibration curve for each element, a graph of mean absorbance against corresponding concentrations of the standard solutions was plotted. In all cases, the graphs were linear; the best fitting straight line was obtained by using Microsoft Excel computer software (Microsoft Office 2000), which also helped to convert absorbance readings to concentrations of elements in each sample analyzed with better accuracy than manual graphical method.

The programme gave concentrations of the diluted and undiluted samples directly. Concentration values obtained for the diluted samples were corrected by multiplying with the respective dilution factors. The final values were expressed as $\mu\text{g/g}$ dry matter, were recorded. These values were obtained by using the expression below:

$$\text{Elemental content} = a/w \times 1000$$

where, a is the amount of element (mg) in 100 ml sample analyzed

w is the dry weight (g) of the material analyzed

2.6 Preliminary *in vivo* Toxicity

Twenty four mice were divided into 8 groups of three animals each for *in vivo* toxicity. The first three animals were treated with saline and served as controls. The other groups were treated with 450 mg /kg body weight of the extracts. The animals were injected with the extracts daily for 30 days and kept under close observation and fed on standard mice pellets and tap water *ad libitum*. Any animal that died or showed signs of death was sacrificed. The animals that were still alive after 30 days were put to sleep using dry ice and sacrificed. The animals were dissected and pieces of pancreas, heart, kidney, muscle and livers were removed and preserved in 10 % formalin for histological preparation and observation.

2.6.1 Histopathological Examination of Tissues

The tissues were removed from formalin and trimmed and then labeled using a pencil. To remove the excess formalin, the tissues were washed in running water overnight. Dehydration was then done in an automatic tissue processor for 3 hours for

each stage starting from 50%, 70%, 80% then 90%. The sections were then washed in a bath of absolute alcohol twice to ensure that there was no trace of water.

The next step was to clear the alcohol from the sections, which was done twice by using xylene. The next step involved infiltration with paraffin wax which was carried out at 2 °C below the melting point of wax for 3 hours. The tissues were then embedded in molten paraffin wax and allowed to dry. The embedded tissues were then sectioned at 4-5µm thickness and floated in warm water bath to spread out the tissues, which were then attached to clean microscopic slides. After holding in hot oven for at least 15 minutes, the sections were dewaxed in xylene and then stained with haematoxylin and eosin dyes using standard histological procedures. The stained tissues were coverslipped with DPX, dried and examined microscopically for pathological changes.

2.7 Data Analysis

In the *in vivo* hypoglycemic assays, student's t-test was used to evaluate the significance between means of extract treated animals and the diabetic control, insulin control and the normal control. The data was represented as means ± SEM. P<0.05 was considered statistically significant. Instat statistical computer package was used

CHAPTER THREE

RESULTS

3.1 *In Vivo* Hypoglycemic Activity Assay

3.1.1 Effect of *Ficus sycomorus* on Blood Glucose in Alloxan-Induced Diabetic Mice

The percent reductions of blood glucose levels in mice by the aqueous stem bark extract of *Ficus sycomorus* at the three dose levels (50 mg/kg body weight, 100mg/kg body weight and 150 mg/kg body weight) during the 1st hour was 30%, 28 % and 49%, respectively. At this hour the plant extract lowered the blood glucose levels but not to normal ($^*P<0.05$)(Figure 1). The doses, however, significantly lowered blood glucose levels as compared to the diabetic control ($^aP<0.05$). During the 2nd hour the glucose lowering effect by the three dose levels was also observed, as the percentage reduction of blood glucose was 54%, 58% and 61% respectively. The extract lowered blood glucose levels to normal ($^bP> 0.05$). In the 3rd hour the extract lowered blood glucose levels by 59%, 65% and 70% respectively. At this hour the extract lowered blood glucose levels as effectively as insulin ($^bP<0.05$) especially by the 150 mg/kg body weight dose range. The same trend was observed during the 4th hour where the three dose levels lowered blood glucose levels lower than even insulin. The percentage blood glucose reductions were 72%, 73% and 78% respectively ($^bP<0.05$).

The aqueous stem bark extract of *Ficus sycomorus* caused a steady decrease in blood glucose levels in the diabetic control mice during the 1st and 2nd hours and then a steep decrease during the 3rd and 4th hours for all the doses.

Table 1: Effects of *Ficus sycomorus* extracts on blood glucose levels in alloxan induced diabetic mice (mg/dl)

Animal Group	Treatment	0 hr	1 hr	2 hr	3 hr	4 hr
Normal	Saline	59.3±2.6	57.3±1.4	60.5±1.5	56.3±4.5	53.8± 2.2
Diabetic	Saline	177.5± 8.3	192.0±7.6	206.8±5.2	216.0±5.6	225.5± 7.1
Diabetic	Insulin 1IU/kgbw	139.5±11.6	51.0±2.3	48.3±3.0	51.8±1.0	54.8±1.4
Diabetic	<i>F.sycomorus</i> 50mg/kgbw	178.0±20.1	133.3±3.4 ^{b*}	80.3±3.8 ^{ab*}	70.3±6.2 ^{ab}	45.8±1.8 ^{ab*}
Diabetic	<i>F.sycomorus</i> 100mg/kgbw	156.8±10.4	112.5±4.6 ^{ab*}	65.0±3.2 ^{ab}	55.0±3.4 ^a	43.5±2.0 ^{ab*}
Diabetic	<i>F.sycomorus</i> 150mg/kgbw	166.8±13.5	85.3±2.8 ^{ab*}	64.3±2.3 ^{ab}	50.0±2.9 ^a	36.4±1.6 ^{ab*}

*P<0.05 with respect to normal control; ^aP<0.05 with respect to diabetic control; ^b P<0.05 with respect to insulin. The data was analyzed using student's t- test. All the results were expressed as mean ± SEM. All blood glucose levels were recorded in mg/dl.

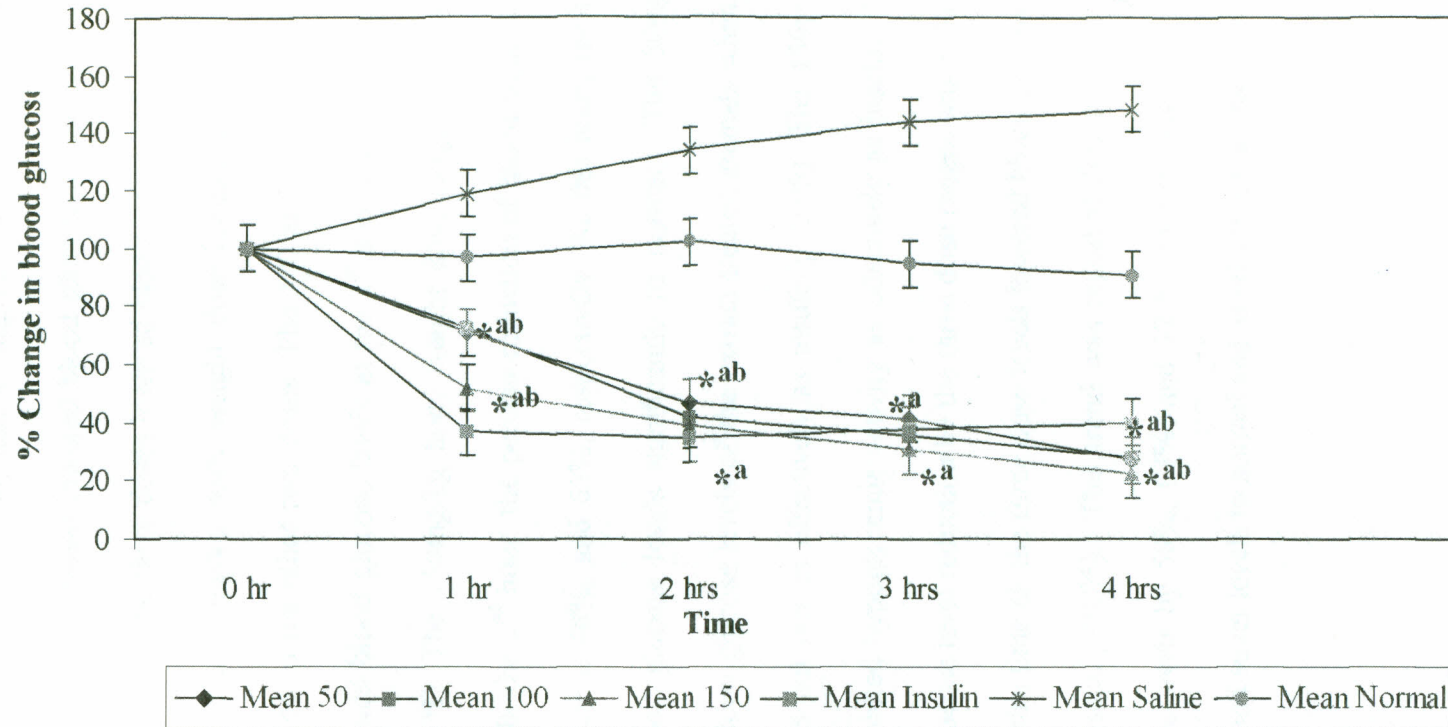


Figure 1: Percentage reduction in blood glucose by varying doses of *Ficus sycomorus* in diabetic mice
 *P<0.05 with respect to normal control; ^aP<0.05 with respect to diabetic control; ^bP<0.05 with respect to insulin.
 The data was analyzed using student's t-test

3.1.2 Effect of *Caesalpinia volkensii* on blood glucose in alloxan-induced diabetic mice

At the three dose levels (50, 100, and 150mg/kg body weight) of the aqueous leave extracts of *Caesalpinia volkensii* lowered blood glucose levels appreciably (Table 2; Figure 2). In the first hour, the three doses lowered blood glucose levels by 5%, 38%, and 51%, respectively. The 50mg/kg body weight dose insignificantly lowered blood glucose levels as opposed to the other two doses. The 100 and 150 mg/kg body weight doses significantly lowered blood glucose levels at the 1st hour but not as effectively as insulin (^a $p < 0.05$; ^b $p < 0.05$). The 150mg/kg body weight dose range lowered the blood sugar level to normal. In the 2nd hour, the percent reduction of blood sugar levels at the three dose levels was 29%, 48%, and 63%, respectively. At this hour, the two higher doses lowered the blood glucose levels significantly to normal. The 50mg/kg body weight dose lowered blood glucose levels of the extract treated animals when compared to the diabetic controls but not as effectively as insulin* $p < 0.05$). The 150mg/kg body weight dose range exhibited hypoglycemic activity as effectively as insulin. During the 3rd hour, the percent glucose level reduction by the three dose ranges was 63%, 54%, and 69%, respectively. At this time all the three dose levels lowered blood glucose to normal and as effectively as insulin^b $p < 0.05$). This trend was repeated during the 4th hour, the extract lowered glucose levels by 58%, 63%, and 72 %, respectively. Here, the three dose ranges lowered blood sugar levels to normal and as effectively as insulin^b $p < 0.05$).

Table 2: Effects of *Caesalpinia volkensii* extract on blood glucose levels in alloxan induced diabetic mice (mg/dl)

Animal Group	Treatment	0 hr	1 hr	2 hr	3 hr	4 hr
Normal	Saline	59.8±1.9	56.5±2.0	56.0±2.4	54.8±1.1	54.8±3.8
Diabetic	Saline	130.0±3.5	142.8±3.8	181.3±5.3	195.8±3.6	210.0±3.1
Diabetic	Insulin 1IU/kgbw	135.0±28.1	50.3±6.2	54.0±1.4	54.5±1.3	51.8±2.7
Diabetic	<i>C. volkensii</i> 50mg/kgbw	140.5±19.7	123.3±12.3 ^{b*}	93.3±6.2 ^{ab*}	49.8±0.3 ^{ab*}	56.3±2.5 ^a
Diabetic	<i>C. volkensii</i> 100mg/kgbw	145.5±17.8	84.8±7.7 ^{ab*}	72.0±5.0 ^{ab*}	63.8±3.1 ^{ab*}	52.3±1.1 ^a
Diabetic	<i>C. volkensii</i> 150mg/kgbw	171.0±16.1	86.3±12.2 ^{ab}	63.5±6.4 ^a	53.0±2.9 ^a	47.5±2.2 ^a

*P<0.05 with respect to normal control; ^aP<0.05 with respect to diabetic control; ^b P<0.05 with respect to insulin. The data was analyzed using student's 't'- test. All the results were expressed as mean ± SEM. All blood glucose levels were recorded in mg/dl.

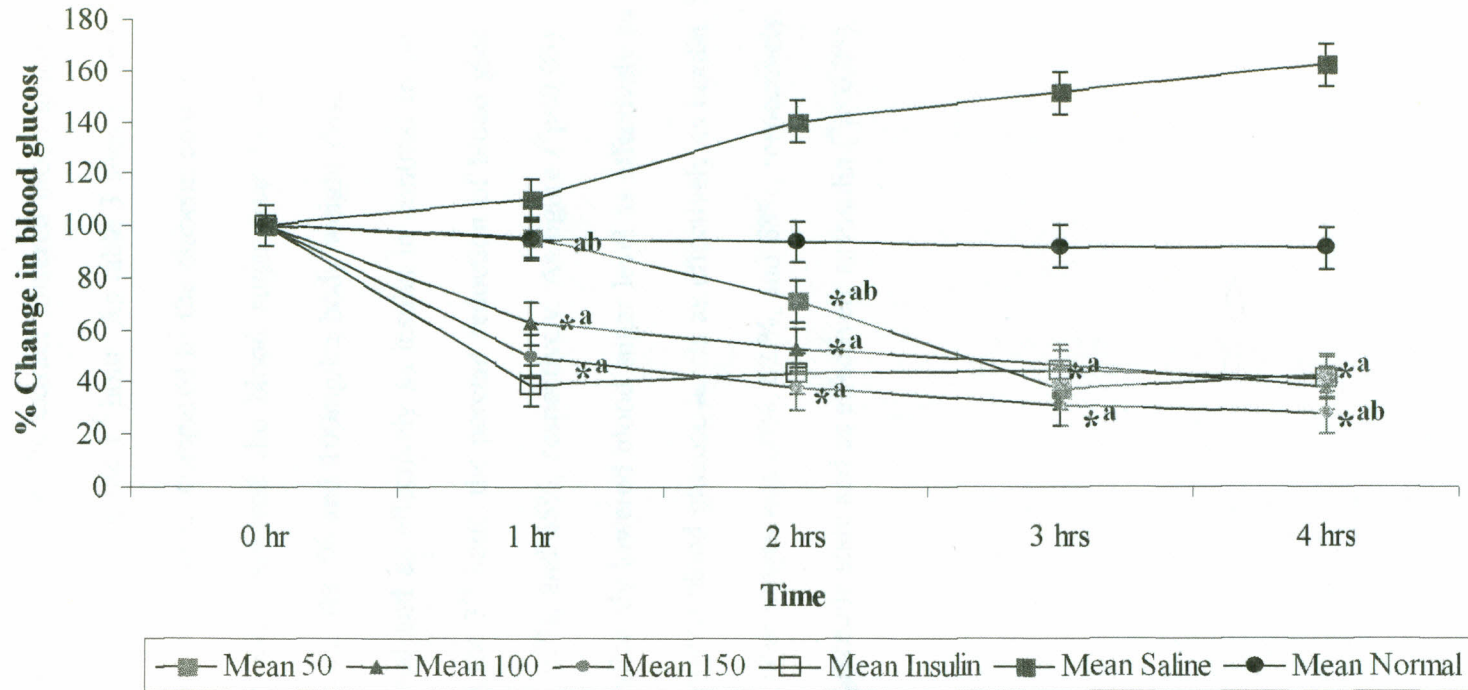


Figure 2: Percentage reduction in blood glucose by varying doses of *Caesalpinia volkensii* in diabetic mice
 *P<0.05 with respect to normal control; ^aP<0.05 with respect to diabetic control; ^bP<0.05 with respect to insulin. The data was analyzed using student's t- test

3.1.3 Effect of *Carissa edulis* on Blood Glucose in Alloxan-Induced Diabetic Mice

The aqueous root bark extract of *Carissa edulis* was also hypoglycemic (Table 3; Figure 3). The three dose ranges of the extract lowered blood glucose levels by 23%, 42%, and 19%, respectively during the 1st hour. As table 3 shows, the three dose ranges lowered blood glucose levels when compared to the diabetic control (^a $p < 0.05$). In the 2nd hour, the three dose levels lowered the blood sugar level by 48%, 60%, and 28%, respectively. At this hour, the 50 and 100mg/kg body weight dose range lowered blood glucose levels to normal and as effectively as insulin in contrast to the 150mg/kg body weight dose range. In the 3rd hour, the percent reduction of blood glucose levels by the three doses was 53%, 62%, and 65%, respectively. As figure (^b $p < 0.05$). 3 shows, all the three dose ranges significantly lowered blood sugar level as effectively as insulin. During 4th hour the extract lowered blood glucose levels as effectively as insulin. The percentage reduction by the three dose ranges was 60%, 62 %, and 68%, respectively. The three dose levels restored normoglycemic state and as effectively as insulin (^b $p < 0.05$).

Table 3: Effects of *Carissa edulis* extract on blood glucose levels in alloxan induced diabetic mice (mg/dl)

Animal Group	Treatment	0 hr	1 hr	2 hr	3 hr	4 hr
Normal	Saline	62.0±3.2	63.0±3.2	55.3±3.4	55.8±1.9	55.3±2.1
Diabetic	Saline	156.8±1.9	164.3±2.6	173.3±4.3	179.0±5.0	189.8±4.4
Diabetic	Insulin 1IU/kgbw	162.3±5.2	52.5±1.3	49.8±0.9	50.5±1.0	51.8±0.9
Diabetic	<i>C.edulis</i> 50mg/kgbw	126.0±0.8	96.5±4.9 ^{ab*}	65.8±10.0 ^a	59.8±9.5 ^a	50.0±6.1 ^a
Diabetic	<i>C.edulis</i> 100mg/kgbw	127.5±13.8	74.5±13.3 ^a	50.8±0.8 ^a	47.8±1.7 ^{a*}	46.8±2.9 ^a
Diabetic	<i>C.edulis</i> 150mg/kgbw	159.3±16.8	132.0±21.1 ^{b*}	117.8±19.6 ^{ab*}	55.3±2.8 ^a	51.3±2.3 ^a

*P<0.05 with respect to normal control; ^aP<0.05 with respect to diabetic control; ^bP<0.05 with respect to insulin. The data was analyzed using student's 't'- test. All the results were expressed as mean ± SEM. All blood glucose levels were recorded in mg/dl.

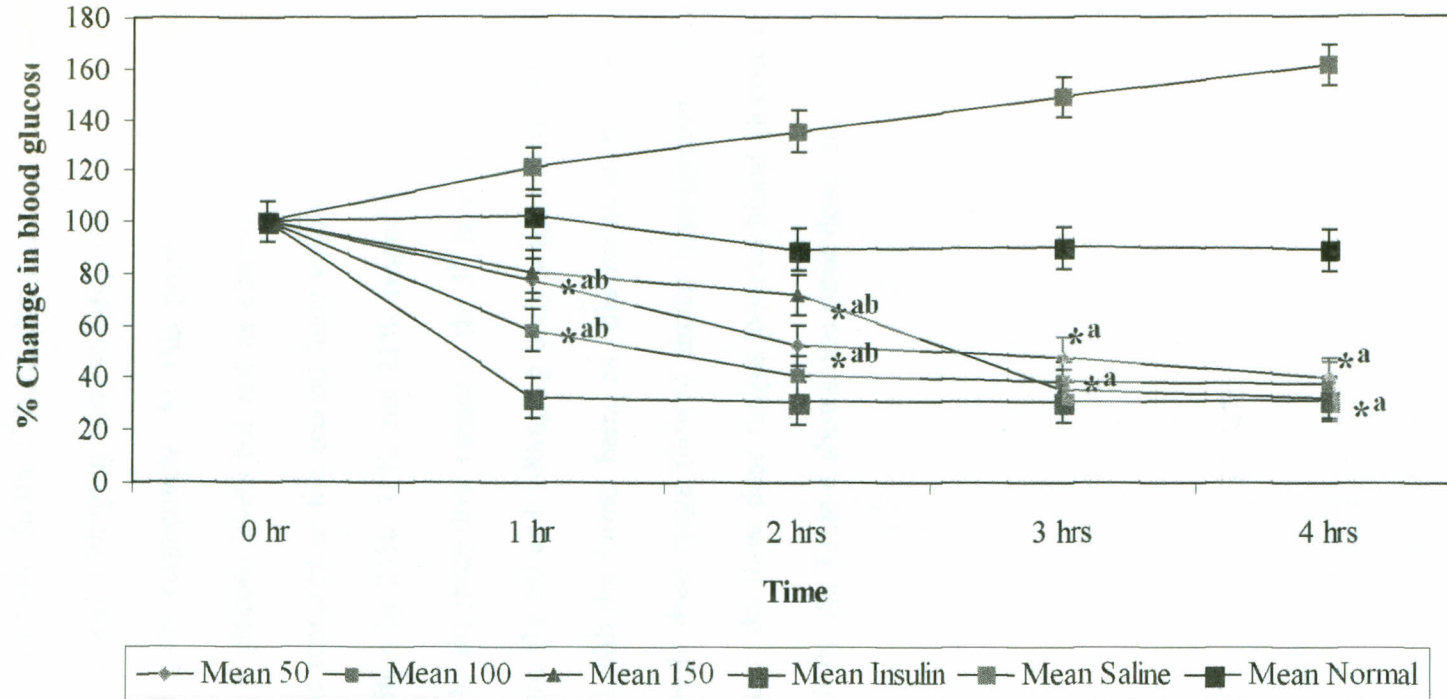


Figure 3: Percentage reduction in blood glucose by varying doses of *Carrisa edulis* in diabetic mice
 *P<0.05 with respect to normal control; ^aP<0.05 with respect to diabetic control; ^bP<0.05 with respect to insulin. The data was analyzed using student's t- test.

3.1.4 Effect of *Kleinia squarrosa* on Blood Glucose in Alloxan-Induced Diabetic Mice

Table 4 and Figure 4 show the pattern of blood glucose reduction by the aqueous stem bark extracts of *Kleinia squarrosa*. In the 1st hour after administration of the extract at the three dose levels (50,100, and 150mg/kg body weight), the blood sugar level was lowered by 28%, 29%, and 14% respectively. At this point, the three dose ranges significantly lowered the blood glucose levels but not as effectively as insulin (^ap<0.05; ^bp<0.05). The same trend was repeated in the second hour where the three dose levels produced hypoglycemic condition by 23%, 41%, and 21%, respectively. The percentage decrease in blood sugar level by the three dose ranges in the 3rd hour was 54%, 51%, and 32% respectively. At this hour, the 50 and 100mg/kg body weight dose range lowered blood glucose level to normal with the former being as effective as insulin. On the other hand, the 150mg/kg body weight dose range showed similar hypoglycemic action as in the second hour. In the 4th hour, the three dose ranges lowered blood glucose levels by 45%, 60%, and 32%, respectively. As Table 4 shows, the three dose ranges lowered the blood sugar levels to normal.

Table 4: Effects of *Kleinia squarrosa* extract on blood glucose levels in alloxan induced diabetic mice (mg/dl)

Animal Group	Treatment	0 hr	1 hr	2 hr	3 hr	4 hr
Normal	Saline	68.3±3.2	65.5±1.9	62.3±2.7	65.3±0.3	67.5± 1.0
Diabetic	Saline	177.5±9.5	178.3±10.2	182.5±12.6	191.3±13.3	199.8± 14.9
Diabetic	Insulin 1IU/kgbw	123.5±15.2	47.3±8.3	49.8± 6.2	54.3± 2.3	62.5± 1.9
Diabetic	<i>K.squarrosa</i> 50mg/kgbw	142.0±14.6	102.9±15.0 ^{ab*}	112.3±12.9 ^{ab*}	62.8±6.3 ^a	75.3± 5.5 ^a
Diabetic	<i>K.squarrosa</i> 100mg/kgbw	161.0 ±16.3	114.8±9.4 ^{ab*}	92.8±3.3 ^{ab*}	76.3±4.7 ^{ab}	61.0± 7.5 ^a
Diabetic	<i>K.squarrosa</i> 150mg/kgbw	151.5 ±6.4	130.3±4.1 ^{ab*}	120.8±11.7 ^{ab*}	103.5±8.5 ^{ab*}	96.2± 6.2 ^{ab}

*P<0.05 with respect to normal control; ^aP<0.05 with respect to diabetic control; ^b P<0.05 with respect to insulin. The data was analyzed using student's 't'- test. All the results were expressed as mean ± SEM. All blood glucose levels were recorded in mg/dl.

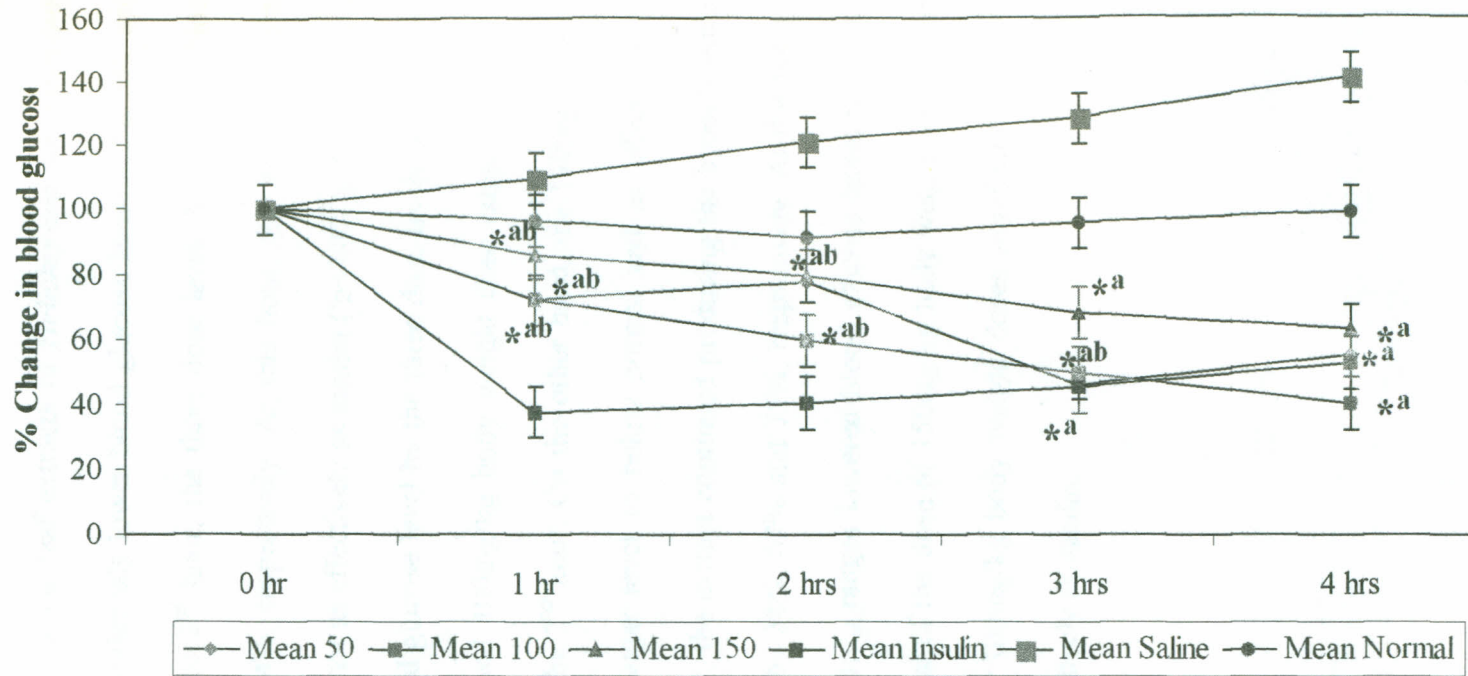


Figure 4: Percentage reduction in blood glucose by varying doses of *Kleinia squarrosa* in diabetic mice
 * $P < 0.05$ with respect to normal control; ^a $P < 0.05$ with respect to diabetic control; ^b $P < 0.05$ with respect to insulin. The data was analyzed using student's t- test.

3.1.5 Effect of *Helichrysum odoratissimum* on blood glucose in alloxan-induced diabetic mice

In the 1st hour, the aqueous leaf extracts of *Helichrysum odoratissimum* at the three dose ranges did not significantly lower blood glucose levels (-2%, 5%, and -11%, respectively)(Figure 5). In the 2nd hour, the three dose levels lowered the blood sugar levels by 30%, 54%, and 54%, respectively. At this hour, the extract lowered blood glucose levels to normal but not as effectively as insulin (^ap<0.05; ^bp<0.05). In the 3rd hour, the percent reduction of blood glucose level by the three dose levels was 26%, 62%, and 70%, respectively. The 50 and 100mg/kg body weight dose range lowered the blood glucose level as they did in the 2nd hour. On the other hand, the 150mg/kg body weight dose range lowered blood sugar level to below normal and as effectively as insulin (Figure 5). In the fourth hour, the extract continued producing the hypoglycemic activity in a dose dependent manner by 38%, 67% and 75%, respectively. At this point, the 100 and 150mg/kg body weight dose ranges lowered blood glucose levels to below normal and the hypoglycemic potential of the dose of 150mg/kg body weight was comparable to that of insulin. The 50 and 100mg/kg body weight doses significantly lowered blood glucose levels but not as effectively as insulin.

Table 5: Effects of *H. odoratissimum* extract on blood glucose levels in alloxan induced diabetic mice (mg/dl)

Animal Group	Treatment	0 hr	1 hr	2 hr	3 hr	4 hr
Normal	Saline	67.5±4.0	68.0± 2.9	63.8±2.7	59.5±1.6	63.8± 2.4
Diabetic	Saline	155.8±11.0	171.0±9.4	188.0±14.3	200.3±14.8	221.8± 15.9
Diabetic	Insulin 1IU/kgbw	191.0±7.5	59.5±1.9	44.8±2.4	39.3±2.4	37.0± 1.4
Diabetic	<i>H.odoratissimum</i> 50mg/kgbw	121.0±12.1	122.3±23.8 ^{b*}	83.5±10.0 ^{ab}	93.3±21.9 ^{ab}	77.3± 4.3 ^{ab*}
Diabetic	<i>H.odoratissimum</i> 100mg/kgbw	175.0±24.9	170.5±29 ^{b*}	79.8±10.0 ^{ab}	58.8±3.6 ^{ab}	54.5± 2.7 ^{ab*}
Diabetic	<i>H.odoratissimum</i> 150mg/kgbw	150.5±5.4	167.0± 4.6 ^{b*}	69.8±8.1 ^{ab}	45.5±3.6 ^{a*}	38.8± 1.1 ^{a*}

*P<0.05 with respect to normal control; ^aP<0.05 with respect to diabetic control; ^b P<0.05 with respect to insulin. The data was analyzed using student's 't'- test. All the results were expressed as mean ± SEM. All blood glucose levels were recorded in mg/dl.

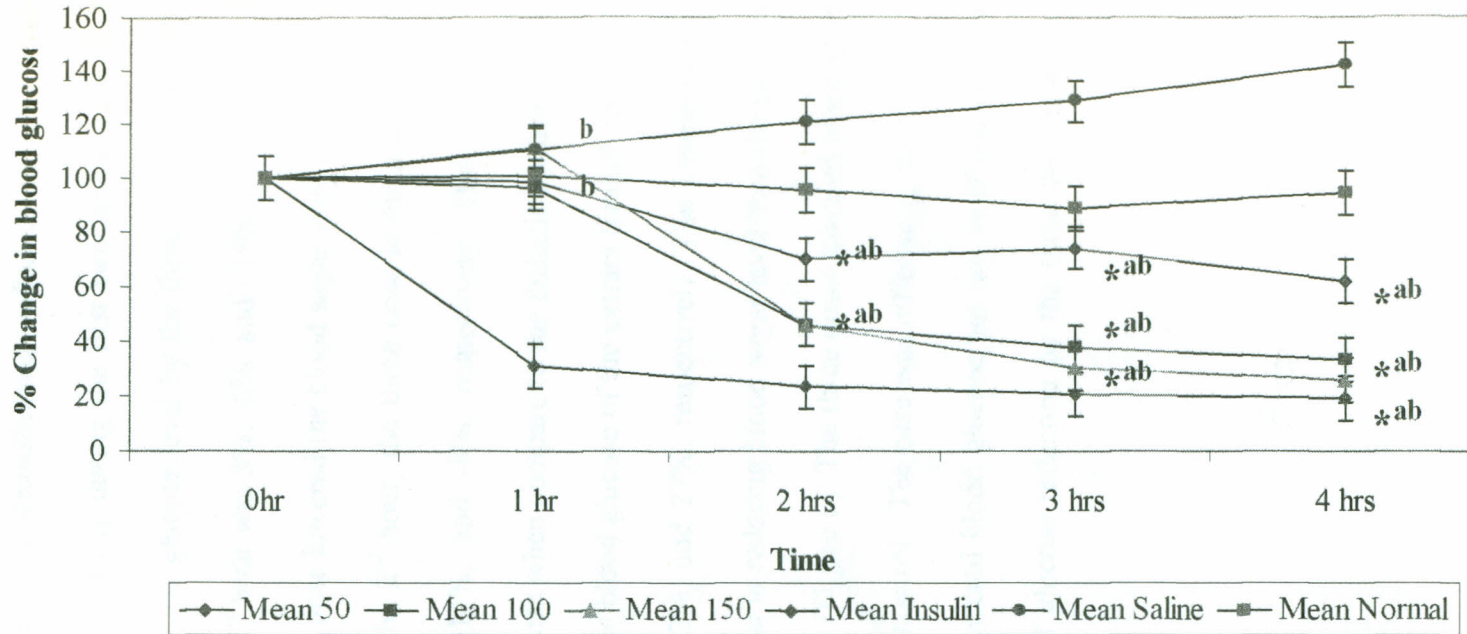


Figure 5: Percentage reduction in blood glucose by varying doses of *H. odoratissimum* in diabetic mice
 *P<0.05 with respect to normal control; ^aP<0.05 with respect to diabetic control; ^bP<0.05 with respect to insulin. The data was analyzed using student's t- test

3.1.6 Effect of aqueous leaf extracts of *Azadirachta indica* on blood glucose in alloxan-induced diabetic mice

The aqueous leaf extracts of *Azadirachta indica* at all dose levels did not appreciably show hypoglycemic activity during the first two hours (Table 6; Figure 6). The percent reduction of the blood glucose level by the three dose ranges (50, 100, and 150mg/kg body weight) in the 1st hour was 3%, -6%, and -15%, respectively. Thus at this hour, the 50mg/kg body weight dose lowered the blood sugar level when compared to the diabetic control (^ap<0.05). In the 2nd hour, the three dose levels insignificantly lowered blood glucose level by 6%, 12%, and -8%, respectively. The 50 and 100 mg/kg bodyweight lowered blood glucose when compare to the diabetic control (^ap<0.05). In the 3rd hour, the percent reduction in blood glucose of the extract treated diabetic mice by the three dose ranges was 22%, 22%, and 27%, respectively. The 150mg/kg body weight dose range had a higher potential in reducing blood sugar level than the two lower doses, contrary to the 1st and 2nd hours (Figure 6). The three doses lowered blood glucose levels when compared to the diabetic control. The case was different in the 4th hour where the three dose levels significantly lowered blood glucose but not as effectively as insulin .At this hour, the percentage blood glucose reduction by the three dose levels was 43 %, 26%, and 39%, respectively.

Table 6: Effects of *Azandiracta indica* extract on blood glucose levels in alloxan induced diabetic mice (mg/dl)

Animal Group	Treatment	0 hr	1 hr	2 hr	3 hr	4 hr
Normal	Saline	61.8±3.4	57.3±2.3	59.5±2.1	51.8±2.8	53.5±2.3
Diabetic	Saline	157.8±4.1	176.0±6.6	192.5±8.0	207.8±4.4	216.3±4.1
Diabetic	Insulin 1IU/kgbw	164.0±3.0	52.0±2.6	55.5±2.0	54.3±0.6	52.3±1.3
Diabetic	<i>A. indica</i> 50mg/kgbw	169.0±8.0	164.5±10.3 ^{b*}	159.0±9.7 ^{ab*}	133.0±6.8 ^{ab*}	96.8±2.3 ^{ab*}
Diabetic	<i>A. indica</i> 100mg/kgbw	150.8±7.6	160.0±9.1 ^{b*}	133.8±10.5 ^{ab*}	116.3±10.4 ^{ab*}	111.0±3.1 ^{ab*}
Diabetic	<i>A. indica</i> 150mg/kgbw	133.8±10.0	151.3±5.4 ^{ab*}	143.3±7.3 ^{ab*}	103.0±26.1 ^{ab*}	82.5±5.1 ^{ab*}

*P<0.05 with respect to normal control; ^aP<0.05 with respect to diabetic control; ^bP<0.05 with respect to insulin. The data was analyzed using student's 't'- test. All the results were expressed as mean ± SEM. All blood glucose levels were recorded in mg/dl.

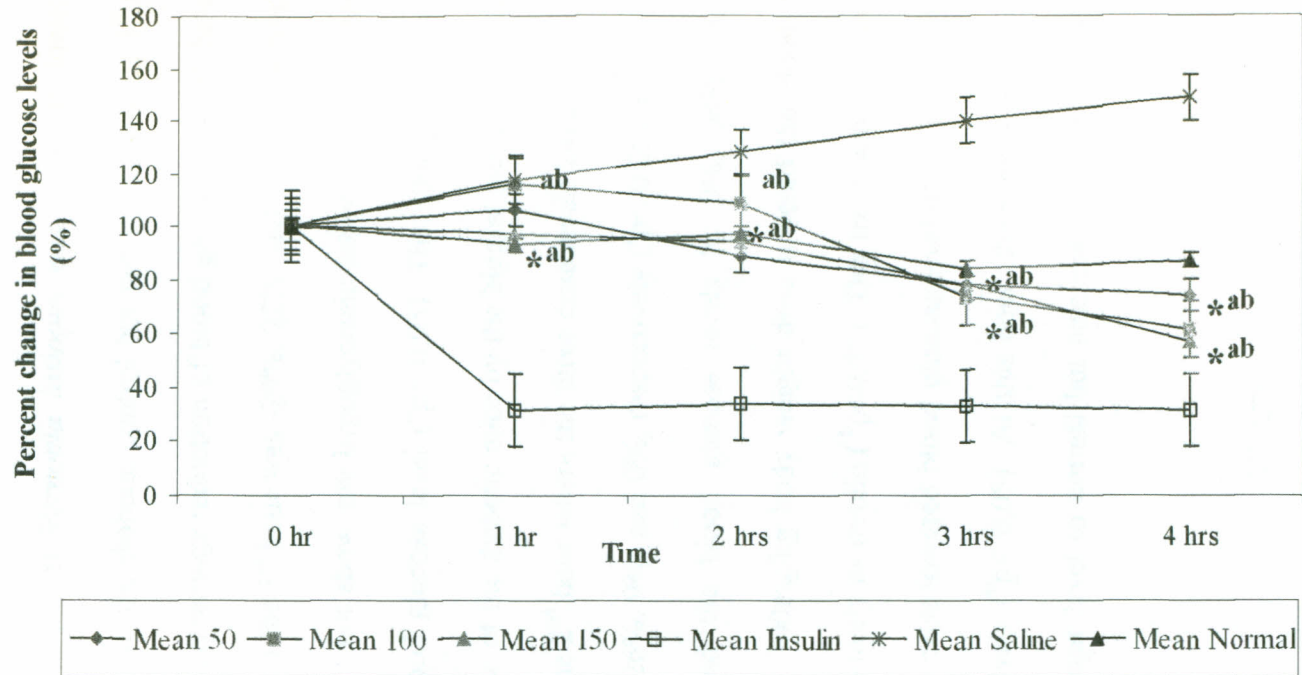


Figure 6: Percentage reduction in blood glucose by varying doses of *Azandiracta indica* in diabetic mice
 *P<0.05 with respect to normal control; ^aP<0.05 with respect to diabetic control; ^bP<0.05 with respect to insulin. The data was analyzed using student's t- test

3.1.7 Effect of aqueous leaves extract of *Vernonia lasiopus* on blood glucose in alloxan-induced diabetic mice

The aqueous leaves extract of *Vernonia lasiopus* was shown to lower blood glucose levels when compared to the diabetic control but not significantly at 1st and 2nd hour (Table 7; Figure 7). The percentage reduction of blood glucose level of the diabetic mice by the three dose ranges in the 1st hour was -29%, 22%, and 11%, respectively. The 50mg/kg body weight dose did not show any hypoglycemic activity and the other doses insignificantly lowered the blood glucose level (^bp< 0.05). The 100mg/kg body weight dose lowered the blood glucose of the diabetic mice by the biggest percentage (Figure 7). The same trend continued in the 2nd hour where the three dose levels lowered blood sugar level of the diabetic mice by -26%, 9%, and 0%, respectively (^ap< 0.05). In the 3rd hour, the three dose levels lowered the blood glucose levels by 9%, 64%, and 20%, respectively. At this hour, the 100mg/kg body weight dose lowered the blood glucose level to normal but not as effectively as insulin (^bp<0.05). This trend was the same in the 4th hour where the three dose levels lowered blood glucose level of the diabetic mice by 41%, 59%, and 28%, respectively (^bp< 0.05). At this hour, the dose level of 50mg/kg body weight lowered blood sugar level to normal but also not as effectively as insulin.

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Table 7: Effects of *Vernonia lasiopos* extract on blood glucose levels in alloxan induced diabetic mice (mg/dl)

Animal Group	Treatment	0 hr	1 hr	2 hr	3 hr	4 hr
Normal	Saline	68.0±3.5	65.8±1.7	65.0±1.7	62.0±1.7	58.5± 1.0
Diabetic	Saline	201.3±6.4	216.5±7.6	226.8±6.9	244.0±7.5	260.3± 13.3
Diabetic	Insulin 1IU/kgbw	186.8±8.5	64.0±3.9	56.3±1.8	51.0±1.8	43.5± 2.6
Diabetic	<i>V.lasiopus</i> 50mg/kgbw	136.5±10.0	174.0±2.7 ^{ab*}	168.8± 11.3 ^{ab*}	119.3±20.9 ^{ab*}	78.5± 11.4 ^{ab}
Diabetic	<i>V.lasiopus</i> 100mg/kgbw	196.5±4.9	157.8±9.1 ^{ab*}	179.0±6.4 ^{ab*}	71.8±9.5 ^{ab}	81.3± 12.0 ^{ab}
Diabetic	<i>V.lasiopus</i> 150mg/kgbw	195.5±21.0	177.0±25.1 ^{b*}	200.8±33.1 ^{b*}	158.3±25.8 ^{ab*}	141.0± 21.1 ^{ab*}

*P<0.05 with respect to normal control; ^aP<0.05 with respect to diabetic control; ^b P<0.05 with respect to insulin. The data was analyzed using student's 't'- test. All the results were expressed as mean ± SEM. All blood glucose levels were recorded in mg/dl.

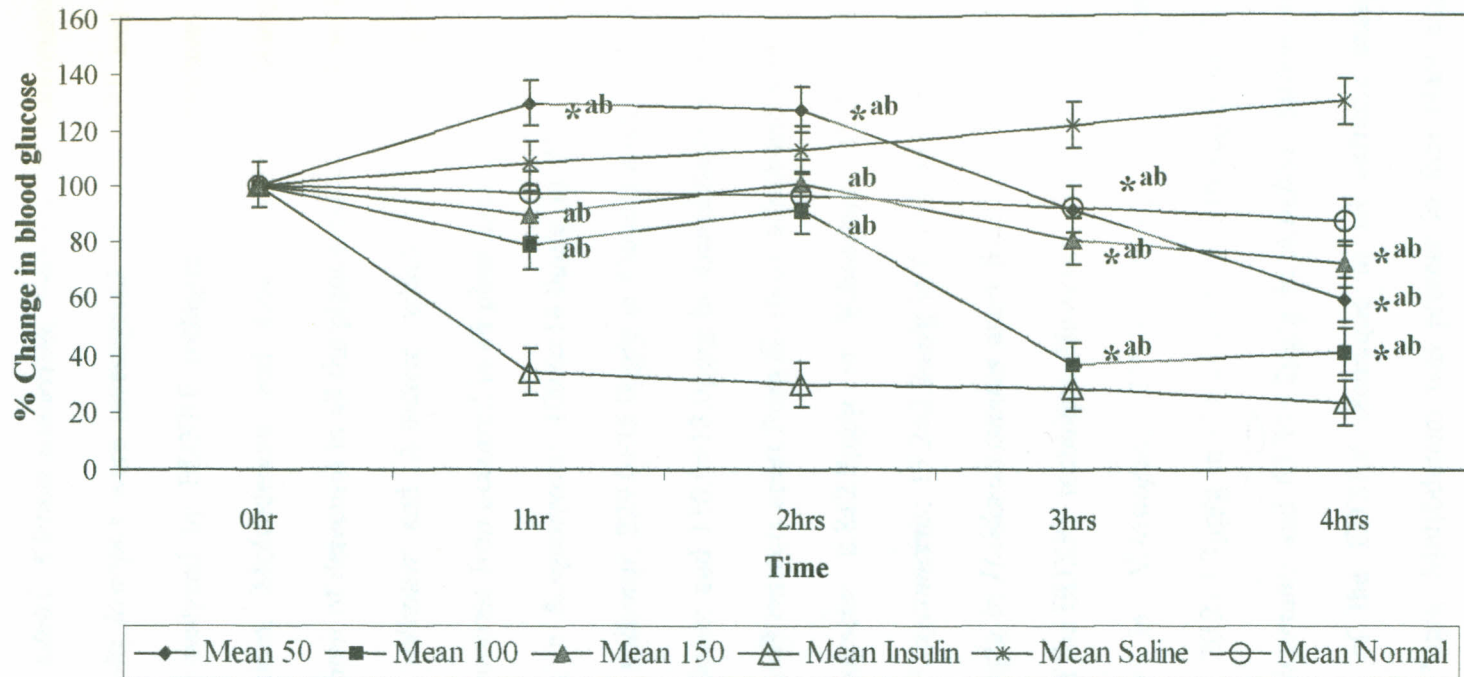


Figure 7: Percentage reduction in blood glucose by varying doses of *Vernonia lasiopus* in diabetic mice
 *P<0.05 with respect to normal control; ^aP<0.05 with respect to diabetic control; ^bP<0.05 with respect to insulin. The data was analyzed using student's t- test.

3.2 Trace Metal Analysis

The different trace elements in the aqueous extracts of *Ficus sycomorus*, *Caesalpinia volkensii*, *Carissa edulis*, *Kleinia squarrosa*, *Helichrysum odoratissimum*, *Azandiracta indica* and *Vernonia lasiopus* were investigated using EDXRF and AAS technique. The trace elements analyzed by EDXRF technique were Manganese, Iron, Nickel, Copper, Zinc, Strontium, Molybdenum and Lead (Table 8). Manganese concentrations were below the limit of detection in all the plant extracts apart from in the aqueous leave extracts of *C.volkensii* and *A.indica*, which had $8.4\pm 1.2\mu\text{g/g}$ and $9.41\pm 0.3\mu\text{g/g}$, respectively. In contrast Iron occurred in all plant extracts: $103.0\pm 9.5\mu\text{g/g}$ in *V.lasiopus*; $365.0\pm 34.0\mu\text{g/g}$ in *K.squarrosa*; $109.0\pm 18.0\mu\text{g/g}$ in *Ficus sycomorus*; $210.0\pm 27.0\mu\text{g/g}$ in *Caesalpinia volkensii*; $229.0\pm 35.0\mu\text{g/g}$ in *Carissa edulis*; $151.0\pm 14.0\mu\text{g/g}$ in *Helichrysum odoratissimum*; and $110.0\pm 18.0\mu\text{g/g}$ in *Azandiracta indica*. Nickel was below the detection limit in all extracts apart from *C.edulis*, which had $8.8\pm 1.5\mu\text{g/g}$. Copper was in six plant extracts: $8.8\pm 2.2\mu\text{g/g}$ in *V.lasiopus*; $35.3\pm 2.7\mu\text{g/g}$ in *K.squarrosa*; $11.9\pm 2.8\mu\text{g/g}$ in *F. sycomorus*; $13.2\pm 2.2\mu\text{g/g}$ in *C.volkensii*; $18.5\pm 2.4\mu\text{g/g}$ in *Carissa edulis*; and $7.9\pm 0.8\mu\text{g/g}$ in *H.odoratissimum* apart from in *A.indica* where it was below the detection limit of the EDXRF technique. However, Zinc was present in all plant extracts: $61.5\pm 4.1\mu\text{g/g}$ in *V.lasiopus*; $235.0\pm 21.0\mu\text{g/g}$ in *K.squarrosa*; $79.0\pm 11.5\mu\text{g/g}$ in *F.sycomorus*; $20.2\pm 3.3\mu\text{g/g}$ in *C.volkensii*; $40.5\pm 6.1\mu\text{g/g}$ in *C.edulis*; and $78.6\pm 6.9\mu\text{g/g}$ in *H.odoratissimum*; and $65.3\pm 4.2\mu\text{g/g}$ in *A.indica*. Strontium was below the limit of detection of the EDXRF technique in all extracts except in *F.sycomorus* which had $2.8\pm 0.7\mu\text{g/g}$. Molybdenum was present in four plant extracts:

24.4±2.8µg/g in *C.volkensii*; 6.6±1.1µg/g in *C.edulis*; 7.6±0.7µg/g in *K.squarrosa*; and 1.8µg/g in *A.indica* but below the limit of detection by the EDXRF technique in extracts from *F.sycomorus*, *H.odoratissimum*, and *V.lasiopus*. Lead was in all plant extracts in low concentrations: 6.4±0.1µg/g in *C.edulis*; 40.8±3.5 µg/g in *K. squarrosa*; 17.6±1.6µg/g in *V.lasiopus*; 7.3±0.5µg/g in *C.volkensii*; 9.1±0.6µg/g in *F.sycomorus* and 11.8±1.2µg/g in *H.odoratissimum*; and 10.1±0.3µg/g in *A.indica* (Table 8).

Magnesium, Chromium and Vanadium were analyzed by Atomic Absorption Spectrophotometry (AAS). Magnesium concentrations were 471.1±19.1µg/g in *C.edulis*; 846.6±1.9µg/g in *K. squarrosa*; 831.4±3.7µg/g in *V.lasiopus*; 762.1±5.9µg/g in *C.volkensii*; 738.6±3.8µg/g in *F.sycomorus* and 950.6±1.1µg/g in *H.odoratissimum*; and 848.3±0.8µg/g in *A.indica* while those for chromium were 112.6±63.0µg/g in *C.edulis*; 100.0µg/g in *K.squarrosa*; 112.6±6.3µg/g in *V.lasiopus*; 105.1±2.9µg/g in *C.volkensii*; 103.8±2.4µg/g in *F.sycomorus* and 105.1±2.9µg/g in *H.odoratissimum*; and 103.8±1.3µg/g in *A.indica*. Vanadium concentrations were below the limit of detection by ASS in all the plant extracts.

Table 8: Trace metals present in the hypoglycemic plants as analyzed by EDXRF ($\mu\text{g/g}$)

Elements	Plants						
	<i>F.sycomorus</i>	<i>C. volkensii</i>	<i>C. edulis</i>	<i>K. squarrosa</i>	<i>H. odoratissimum</i>	<i>A. indica</i>	<i>V. lasiopus</i>
Mn ↓	<5	8.4±1.2	<5	<5	<5	9.4±0.2	<5
Fe	109.0±18.0	210.0±27.0	229.0±35.0	365.0±34.0	151.0±14	110.0±18.0	103.0±9.5
Ni	<5	<5	8.8±1.5	<5	<5	<5	<5
Cu	11.9±2.8	13.2±2.2	18.5±2.4	35.3±2.7	7.9±0.8	<5	8.8±2.2
Zn	79.0±11.5	20.2±3.3	40.5±6.1	235.0±21.0	78.6±6.9	65.3±4.2	61.5±4.1
Sr	2.8±0.7	<1	<1	<1	<1	<1	<1
Mo	<5	24.4±2.8	6.6±1.1	7.6±0.7	<1	1.8±0.0	<1
Pb	9.1±0.6	7.3±0.5	6.4±0.1	40.8±3.5	11.8±1.2	10.1±0.3	17.7±1.6

Table 9. Trace metals present in the hypoglycemic plants as analysed by AAS ($\mu\text{g/g}$)

Plants	Elemental Quantity ($\mu\text{g/g}$)		
	Magnesium (Mg)	Chromium (Cr)	Vanadium (V)
<i>Caesalpinia volkensii</i>	762.1 \pm 5.9	105.1 \pm 2.9	<1
<i>Ficus sycomorus</i>	738.6 \pm 3.8	103.8 \pm 2.4	<1
<i>Carissa edulis</i>	471.1 \pm 19.1	112.6 \pm 63.0	<1
<i>Kleinia squarrosa</i>	846.6 \pm 1.9	100.0 \pm 0.0	<1
<i>H. odoratissimum</i>	950.6 \pm 1.1	105.1 \pm 2.9	<1
<i>Azandirecta indica</i>	848.3 \pm 0.8	103.8 \pm 1.3	<1
<i>Vernonia lasiopus</i>	831.4 \pm 3.7	112.6 \pm 6.3	<1

3.3 Classes of Compounds in the aqueous Plant extracts

Phytochemical screening for the aqueous extracts of *Ficus sycomorus*, *Caesalpinia volkensii*, *Carissa edulis*, *Kleinia squarrosa*, *Helichrysum odoratissimum*, *Azandirecta indica*, and *Vernonia lasiopus* was undertaken to determine the class of compounds present in them. Results show that *Ficus sycomorus* plant extract had only saponins and tannins. Sterols, flavonols/flavones or chalcones and flavonoids were present in the leaf extract of *Caesalpinia volkensii*. *Carissa edulis* roots contained flavonols/ chalcones or flavones, flavonoids and tannins. Saponins, flavonols/ flavones or chalcones and tannins were present in *Kleinia squarrosa*. *Helichrysum odoratissimum* leaves had alkaloids, terpenoids and tannins. *Azandirecta indica* plant extract had alkaloids, terpenoids, flavones/ flavonols or chalcones, flavonoids, and both free and bound anthraquinones. *Vernonia lasiopus* leaf extract also contained all the phytochemical compounds tested except free anthraquinones, alkaloids, and terpenoids

Table 10: Phytochemistry of the seven medicinal plants

Classes of compounds	Plants						
	<i>F. sycomorus</i>	<i>C. volkensii</i>	<i>C. edulis</i>	<i>K. squarrosa</i>	<i>H. odoratissimum</i>	<i>A. Indica</i>	<i>V. lasiopus</i>
Alkaloids	-	-	-	-	+	+	-
Sterols	-	+	-	-	-	-	+
Terpenoids	-	-	-	-	+	+	-
Saponins	+	-	-	+	-	-	+
Flavonols/flavones/Chalcones	-	+	+	+	-	+	+
Flavonoids	-	+	+	-	-	+	+
Tannins	+	-	+	+	+	-	+
Free Anthraquinones	-	-	-	-	-	+	-
Bound Anthraquinones	-	-	-	-	-	+	+

Key: +Ve → Compound present

-Ve → Compound absent

3.4 Preliminary *in vivo* toxicity

In vivo toxicity assay was undertaken on 24 mice that were divided into 8 groups of three mice each. The first group of animals was treated with saline and served as controls for the other extract treated groups. The other groups were treated separately with the aqueous extracts of *Ficus sycomorus*, *Caesalpinia volkensii*, *Carissa edulis*, *Kleinia squarrosa*, *Helichrysum odoratissimum*, *Azandiracta indica*, and *Vernonia lasiopus* at a dose of 450mg/kg body weight as explained in section 2.6. The results obtained were:

Ficus sycomorus aqueous stem bark extract caused local irritation to the body tissues at the point of injection. This was evident after histopathological examination of skeletal muscle tissue near the point of injection, which demonstrated by pyrogranulomatous inflammation of the soft tissue. The kidney cells were not damaged but there was mild perirenal steatite. Liver, spleen and heart tissues were normal as there were no signs of pathology.

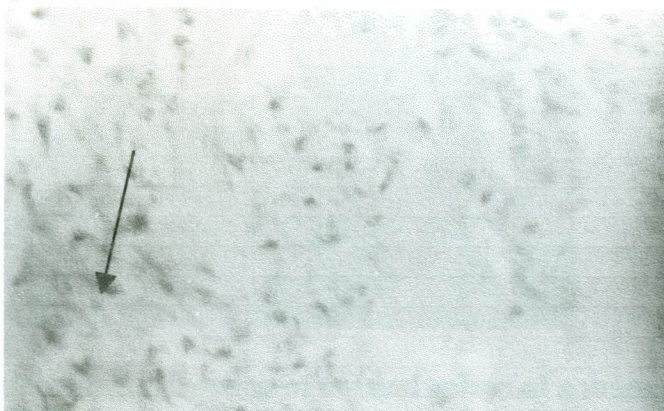


Plate 1: Histological section of skeletal muscle of a mouse treated with an aqueous stem bark extract of *Ficus sycomorus* (450mg/kgbw). Inflammation of muscle tissue (arrow). Exposure time: 30 days Magnification: X400

Aqueous leaf extract of *Caesalpinia volkensii* plant extract had no toxic effects on tissues examined. The liver tissue showed no signs of damage; the hepatocytes were intact apart from mild inflammation in the liver. Kidney examination showed isolated perivascular accumulation of lymphocytes but the renal cells were normal. The spleen and heart tissues showed no signs of pathology.

Aqueous root bark extract of *Carissa edulis* showed no pathology in the liver apart from mild irritation on the liver surface and mild inflammation. Examination of the kidney tissues showed mild perivascular accumulation but the kidney cells were intact. The spleen and the heart tissues were normal.

Animals treated with the leaf extract of *Helichrysum odoratissimum* showed mild perivascular inflammation in the kidneys but the renal cells were intact. The spleen tissues cells were intact apart from mild lymphoid depopulation. The liver had no signs of pathology, the hepatocytes were intact but there was mild perihepatitis. The heart muscle had no pathology.



Plate 2: Histological section of normal spleen of a mouse treated with normal saline. Follicles are densely populated (0.1ml). Exposure time: 30 days. Magnification: X100.

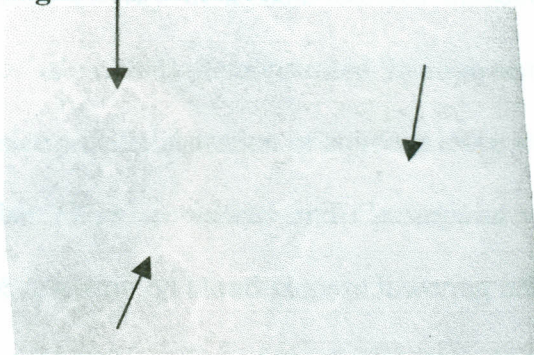


Plate3: Histological section of a spleen of a mouse treated with an aqueous stem bark extract of *Helichrysum odoratissimum* (450mg/kgbw). Reduction in lymphoid population manifested by absence of follicles (arrows) Exposure time: 30 days. Magnification: X100

The body tissues of animals treated with the aqueous leaf extracts of *Azadiracta indica* showed no pathology except mild lymphoid depopulation in the spleen and mild inflammation in the liver tissues.

Vernonia lasiopus leaf extract had no toxicity on body tissues of mice after treatment with the dose 450mg/kg body weight apart from mild inflammation in the liver. The other organs had no pathology.

Animals injected with 450mg/kg body weight of *Kleinia squarrosa* stem bark extract shown no pathological lesions in the organs that were examined.

CHAPTER FOUR

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

4.1 Discussion

The aqueous stem and root bark extracts of *Ficus sycomorus* and *Kleinia squarrosa* and *Carissa edulis* respectively, leaf extracts of *Caesalpinia volkensii*, *Helichrysum odoratissimum*, *Azandiracta indica* and *Vernonia lasiopus* showed hypoglycemic activity in alloxan-induced diabetic mice. Similar work carried out by Tsuneki *et al.* (2004) demonstrated hypoglycemic activity on streptozotocin induced diabetic mice on administration of aqueous leave extracts of *Camellia sinensis*.

That *Ficus sycomorus* and *Caesalpinia volkensii* extracts demonstrated a dose dependent response on blood glucose lowering effect on alloxan induced diabetic mice. A dose dependent hypoglycemic activity was also observed by Jouad *et al.* (2004) on studies carries out on streptozotocin induced diabetic rats orally administered with leave extracts of *Eucalyptus globules*. In another related study Nammi *et al.* (2003) demonstrated a dose dependent hypoglycemic activity on alloxan-induced diabetic rabbits on administration of fresh leave extracts of *Catharanthus roseus* Linn.

Similar results were observed by Aguiyi *et al.* (2000) who demonstrated hypoglycemic activity on normal and alloxan-induced diabetic rats intraperitoneally injected with methanolic extracts of *Ocimum gratissimum* leaves. This indicates that these plant extracts might have been absorbed through the cell lipids membrane through facilitated diffusion. The ions might have been transported in the direction of its electrochemical gradient. This tread is in agreement with expectations seen in

administration of higher concentration of drug. *Ficus sycomorus* and *C. volkensii* also lowered blood sugar to below insulin levels. This suggests the possibility of islet repair or mimicry of insulin action by elements from the extract.

Carissa edulis, *Helichrysum odoratissimum*, *Kleinia squarrosa* and *Vernonia lasiopus* plant extracts demonstrated a non dose dependent response. This trends might suggest that the extract may have been absorbed in the cell system through active transport where at a particular concentration saturation of the extract occurred resulting in the rest of the extract being excreted *Azadirachta indica* did not have appreciable activity during the first three hours but significant activity was observed in the 4th hour. This suggests that the extract might have been a prodrug, which was biotransformed to an active form.

Besides, studies done with ethanolic seed extracts of *Luffa aegyptiaca* and leaves of *Carissa edulis* have been shown to significantly lower blood glucose levels in streptozotocin-induced diabetic rats (EL-fiky *et al.*, 1996). Ozbek *et al.* (2004) also demonstrated a significant blood glucose lowering effect in alloxan- induced diabetic mice by aqueous root extracts of *Rheum ribes*. Ethanolic leaf extracts of *Cassia kleinii* were also shown to lower blood glucose in streptozotocin-induced diabetic rats (Babu *et al.*, 2003).

The hypoglycemic effect of the plants under study can be attributed to alkaloids, sterols, terpenoids, saponins, flavonols, flavones, chalcones, flavonoids, tannins, free anthraquinones and bound anthraquinones observed in these plants. Several studies have shown the hypoglycemic activity of such compounds. Saponins were shown

to lower blood glucose in studies undertaken on ginseng species in alloxan-treated diabetic rats (Kimura and Suzuki, 1985). Besides, flavonols, sterols flavones, and chalcones in *Trigonella foenum graecum* plant extract have been shown to lower blood glucose in diabetic animals (Marles and Farnsworth, 1994; Duke, 1997). Methylhydroxy chalcone polymer (MHCP) from the extract of cinnamon has been shown to increase glucose metabolism of cells 20-fold *in vitro* in the epididymal fat cell assay (Broadhurst, 1997).

Flavonoids present in *Pterocarpus marsupium* bark extracts have been shown to prevent beta cell damage in rats (Chakravarthy *et al.*, 1981). The Ginseng species have terpenoidal components, which cause hypoglycemic effects in streptozotocin rat models (Shapiro and Gong, 2002). The roots of *Abroma augusta* (Linn) contain some alkaloid bases, which have been shown to reduce blood glucose levels in streptozotocin induced diabetic rats (Eshrat and Hussain, 2002). Plant extract containing alkaloids may have lowered blood glucose levels through the mechanism of beta cell regeneration. Alkaloids such as alkaloid 1- ephedrine promote regeneration of pancreatic islets following atrophy, restore the secretion of insulin and promote hypoglycemia (Elloit *et al.*, 2000). *Polygonum multiflorum* plant extracts, which contain anthraquinones, are used in the treatment of peripheral neuropathy, a complication associated with diabetes mellitus (Broadhurst, 1997).

Flavonoids, tannins and saponins present in the aqueous extract of *Boerhavia diffusa* leaves produced non-dose dependent related decrease in blood glucose levels in alloxan induced rats (Chude *et al.* ,2000). Methanolic root extracts of *Clitoria ternatea*

Linn contains flavonoids, steroids, triterpenoids, saponins and tannins which are responsible for the glucose lowering ability in both normal and streptozotocin induced diabetic rats (Boominathan *et al.*, 2004). The methanolic extracts of *Cleome viscosas* Linn and the pentacyclic triterpenoid betulin isolated from chloroform fraction of methanol extract exhibited significant hypoglycemic activity in both normal and streptozotocin induced diabetic rats. The effect was dose dependent (Parimaladevi *et al.*, 2004). Terpenoids from bitter melon (*Momordica charantia* L) are responsible for the plant's hypoglycemic properties. In a clinical trial improved glucose tolerance was observed in 73 % of patients with NIDDM who were administered *M. charantia* fruit juice (HerbalGram, 1997).

Alkaloids such as catharanthine, leurosine sulphate, lochnerine, tetrahydroalstonine, vindoline and vindolinine were shown to lower blood sugar levels (thus easing the symptoms of diabetes). Alkaloids isolated from the Madagascar periwinkle (*Catharanthus roseus* (L.) and marketed in England under the proprietary name Vinculin lower blood glucose in diabetic patients (HerbalGram, 1997). Antidiabetic and antihyperlipidemic effect of *Zizyphus jujuba* in alloxan induced diabetic rats is due to the alkaloid barberine present in the leaves of the plant. The chemical constituents in *Z. jujuba* may have the ability to release insulin from pancreatic β -cells and also have the potential to protect it from alloxan-induced damage in experimental animals (Tiwari and Rao, 2002).

Medicinal herbs used in indigenous medicines for the management of diabetes mellitus contain both organic and inorganic constituents. Some of these inorganic trace

elements possess antidiabetic properties, which could account for the activity of medicinal herbs. The blood glucose lowering effect by the aqueous extracts of the plants of this study could also have been caused by trace elements. Such trace elements were isolated in appreciable amounts from the plants such as Manganese, Copper, Nickel, Magnesium, Zinc, Molybdenum and Chromium present in these plants. It has been shown that Manganese reduces glucose intolerance present in deficiency cases (Keen and Zidenberg-Cherr, 1996). Similarly Copper deficiency is associated with disturbed carbohydrate metabolism. Zinc is involved in all aspects of insulin metabolism: synthesis, secretion and utilization, while Zinc deficiency plays a role in the development of diabetes in humans. Zinc also has a protective effect against beta cell destruction, and has well-known anti-viral effects (Lehman and Spinass, 1996).

Molybdenum stimulates glycolysis and accelerates glycogen degradation in the hepatocytes. It also increases receptor autophosphorylation and phosphorylation of its substrate and augment glucose transport (Li *et al.*, 1995). Magnesium deficiency is the most evident disturbance of metal metabolism in diabetes mellitus. Hypomagnesemia increases the risk of ischemic heart disease and severe retinopathy (Tuvemo and Gebre-Medhin, 1985; Matsumoto, 1994; Singh, 1995; Frank *et al.*, 2000).

Chromium enhances the body's sensitivity to insulin and increases the number of insulin receptors, to enhance receptor binding. It also potentiates insulin action (Anderson *et al.*, 2001). Experimentally, Chromium deficiency is associated with impaired glucose tolerance, which is improved with supplementation (Trow *et al.*, 2000). The chromium, manganese and magnesium salts present in the saltbush *Atriplex halimus*

L., are believed to prevent diabetes from occurring in sand rats who feed regularly on the plant and who have a genetic predisposition to diabetes (Snyder, 1996; HerbalGram, 1997). Studies undertaken by Ravi *et al.* (2004) shown that inorganic trace elements such as zinc, chromium and vanadium which were present in the seeds of *E. jambolana* had hypoglycemic activity in streptozotocin-induced diabetic rats .

The aqueous plants extracts studied did not alter the normal cell structure of the heart, kidney, liver and spleen as indicated. This suggested the safety of these plants when used to manage diabetes mellitus. The inflammation at the site of injection may also be attributed to drug induced reactions (Paumgarten *et al.*, 1990). The surface irritations on the kidney that were present in all plant extracts apart from in *Azadiracta indica* and *Vernonia lasiopus* could also be attributed to tannins and saponins, which were present in these extracts (Diwan, 2000). Saponins and tannins cause hemorrhage and congestion of veins in tissues (Chung *et al.*, 1998; Diwan, 2000). Terpenoids may also have been responsible for the inflammation since terpenoids such as β -myrcene are toxic to the stomach, liver and are highly irritating to the peritoneum (Paumgarten *et al.*, 1990). Alkaloids present in the plant extracts may also have been responsible for the local inflammation. Zeinsteger *et al.* (2003) showed that *Senecio grisebachii* alkaloids such as pyrrolizidine alkaloids (PAS) when metabolized in the liver produce toxic products which cause intense cellular alterations including megalocytosis. Further metabolism of the alkaloids present in the plant extracts may have produced toxic products that caused surface irritations on the kidney.

The range of doses used in this study were within the doses used by Tsuneki *et al.* (2004) and Jouad *et al.* (2004). Tsuneki *et al.* (2004) while examining the hypoglycemic effect of green tea on blood glucose levels used a dose range of 30-300mg/kg body weights in rats. Jouad *et al.* (2004) used a dose range of 150 and 300 mg/kg body weight while evaluating the hypoglycemic activity of aqueous extract of *Eucalyptus globulus* in normal and streptozotocin-induced diabetic rats. Jouad *et al.* (2004) used a dose of 4.5 g/kg body weight of *Eucalyptus globulus* leaf extract to evaluate toxicity of this plant extract.

4.2 Conclusion

All the seven aqueous medicinal plant extracts showed significant antidiabetic property. The results of this study confirm the suitability of *Ficus sycomorus*, *Caesalpinia volkensii*, *Carissa edulis*, *Kleinia squarrosa*, *Helichrysum odoratissimum*, *Azandirecta indica* and *Vernonia lasiopus* extracts for the management of diabetes mellitus. However, the modes of their action are still obscure.

The different trace elements and phytochemical compounds present in these plants were associated with the hypoglycemic activity. Histological responses induced by the extracts indicated no overt pathology attributable to these plants after intraperitoneal administration. This might indicate their low acute toxicity after intraperitoneal administration. Further toxicity tests using the oral route of administration at standardized dosage levels will be needed to comprehensively evaluate the safety of these extracts in animal models after administration through the conventionally used route of treatment for the management of diabetes mellitus. Herbal medicines are complex mixtures of different

compounds that often act in a synergistic manner and exert their full beneficial effect as total extracts. Since herbalists use the extracts in combination for the management of diabetes mellitus, incidences of toxicity are greatly arrested. In this study, the null hypothesis is accepted and the study justified.

4.3 Recommendations

This study could not be exhaustive owing to the time frame within which it was done. For this reason, further studies require to be undertaken. Viz:

- The blood glucose levels were only measured until the fourth hour. It would be desirable to measure the blood glucose levels for longer periods so as to determine the duration of action of the extracts.
- Studies should also be undertaken using other routes of extract administration such as the oral route, which is normally used by herbalists to administer the medicines. This would establish whether the extracts are inactivated by the digestive enzymes.
- Further toxicology tests should be undertaken to determine the safety of the extracts. Tests should be undertaken on other body organs apart from the liver, kidney, spleen and heart. The brain and intestines should also be examined to determine the effects of the extract on the brain cells and gastrointestinal tract. Liver functional tests should be included in the toxicological studies.
- Further studies on healthy animals that are not diabetic but given extracts so that one may be able to establish the mechanism of action of these extracts are required.

- Isolation and structural elucidation of the bioactive compounds would also be desirable.
- Combination therapy should also be undertaken as done by the traditional herbalists to test whether they are still hypoglycemic.
- Further studies should also be undertaken with the same plant species using the same doses from other geographical areas and different seasons to test whether the hypoglycemic activity is geographically and seasonally related.
- Asses the potential of trace elements in the extracts to lower blood glucose *in vitro*

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Parte de la planta	Trace Elements		
	Calcium	Magnesium	Zinc
Flowers	1010	2852	7184
Stems	10	57	02
Leaves	10	50	50
Roots	10	70	30
Whole plant	12	17	12

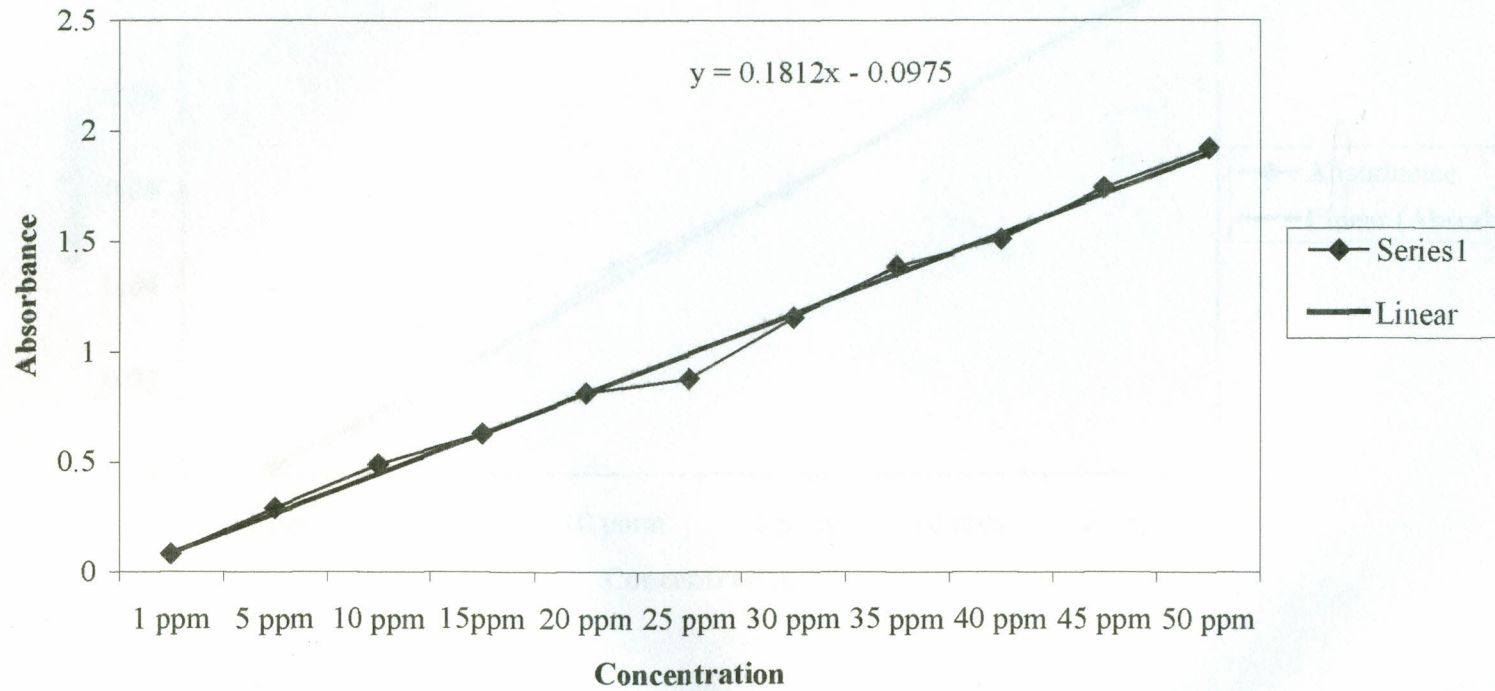
Appendices

Appendix 1: Instrumental conditions for Atomic Absorption Spectrophotometer (AAS)

Parameter	Trace Elements		
	Chromium	Magnesium	Vanadium
Wavelength (nm)	357.9	285.2	318.4
Slit Width (nm)	0.7	0.7	0.2
Lamp Current (mA)	5.0	5.0	5.0
Flow Rate (l/min)			
Air	7.0	7.0	7.0
Acetylene	1.8	1.8	1.8

Appendix 2: Standard Calibration Curve for Magnesium

Concentration (ppm)	1	5	10	15	20	25	30	35	40	45	50
Absorbance	0.08	0.29	0.49	0.63	0.81	0.88	1.16	1.39	1.51	1.72	1.92



Appendix 3: Standard Calibration Curve for Chromium

Concentration (ppm)	1	5	10	15	20	25
Absorbance	0.002	0.018	0.044	0.060	0.080	0.100

