HYPOGLYCAEMIC POTENTIAL OF SOME KENYAN PLANTS USED IN TRADITIONAL MEDICINE IN LAIKIPIA AND MBEERE DISTRICTS AND NAIROBI PROVINCE

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

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DECLARATION

I, Kibiti Mwiti Cromwell, duly declare that this is my original work and has not been presented for a degree in any other university or for any other award.

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DEDICATION

This thesis is dedicated to my father Lawi Kibiti and my mother Deborah M. Kibiti
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ABSTRACT

Diabetes mellitus is a chronic physiological metabolic disorder caused by inherited and/or acquired deficiency in production of insulin by pancreas or by the ineffectiveness of the insulin produced. The prevalence of diabetes in adults globally was estimated to be 4.0% in 1995 and was projected to rise to 5.4% (300 million) by the year 2025. In Kenya, it was estimated to be 1.06% in 2000 and projected to rise to 1.32% by the year 2025. Conventional drug therapy though effective in the management of diabetes mellitus is expensive and has toxic side effects. Herbal medicine would thus provide alternative therapy if effective and less toxic. However, their safety and effectiveness has not been investigated. The aim of this study is therefore to assess whether aqueous leaf extracts of *Aloe secundiflora*, *Olea africana*, *Pentas zanzibarica* and *Rumex abyssinicus*; root extracts of *Terminalia brownii*; root tuber extracts of *Rhoicissus tridentata* and stem bark extracts of *Warburgia salutaris* had in vivo hypoglycaemic activity in alloxan induced diabetic mice. The seven plants were collected from Rift Valley and Eastern provinces using ethno-botanical and pharmacological information obtained from traditional healers. Elemental analysis of the extracts was done using energy dispersive X-ray fluorescence spectrometry and atomic absorption spectrometry. Phytochemical screening of the extracts was done to determine the class of secondary metabolites present in the plant extracts. Histological assessment was done to evaluate the toxicological effects of the extracts on the various organs. Out of the seven plants, six aqueous leaf extracts of *Aloe secundiflora*, *Olea africana* and *Pentas zanzibarica*; root extracts of *Terminalia brownii*; root tuber extracts of *Rhoicissus tridentata* and stem bark extracts of *Warburgia salutaris* showed blood glucose lowering effect in alloxan induced diabetic mice. The elements Cr, Cu, Fe, Pb, Ni, Mg, Mn, Mo, Sr and Zn were present in the plant extracts but in different quantities. Alkaloids, saponins, tannins, terpenoids, sterols, flavonoids and anthraquinones were present in some of the plants. On histological assessment, aqueous leaf extracts of *Olea africana* had no effects on the livers, spleens, hearts, lungs and kidneys. Among the plants with hypoglycaemic activity, *Olea africana* is safe to serve as an alternative therapy for diabetes management. However, though the other plant extracts showed toxicity, they continue to be used to manage diabetes mellitus since the observed toxicity of single plant extracts is reduced by combining extracts from different plants.
Chapter One

Introduction and Literature Review

1.1 Introduction

Diabetes mellitus is a chronic physiological metabolic disorder caused by inherited and/or acquired deficiency in production of insulin by pancreas, or by the ineffectiveness of the insulin produced. Such a deficiency results in increased concentrations of glucose in the blood, which in turn damages many of the body's systems, in particular the blood vessels and nerves (WHO 2002). In humans, it is due to a disorder of the pancreatic β-cells, in which insulin production is either lost or impaired, or from which a defective form of insulin is secreted (Mwanga 2000).

A World Health Organization (WHO) study has shown that an estimated 30 million people worldwide had diabetes (WHO 1985). A decade later, the global burden of diabetes was estimated to be 135 million. The latest WHO estimate for the number of people with diabetes worldwide, in 2000 was 177 million (WHO 2002), and 3.2 million deaths per year was attributed to this disease (WHO 2002). Due to population ageing and further urbanization, this is likely to increase to at least 370 million by 2030 and most of this increase will be in the developing countries (WHO 2002). However, in Kenya, in 1995 it was estimated that, 1.0% of the population had diabetes, and this rose to 1.06% in 2000. It is estimated that by the year 2025, 1.32% of the population will be diabetic (Hilary et al. 1998).

The major factors identified for developing diabetes are inheritance, genetic predisposition, and environmental factors such as nutrition and chemical toxins. Excessive levels of glucagon can also lead to the development of hyperglycaemia (WHO 1985). The currently accepted classification of diabetes mellitus are Type I (Insulin-Dependent) diabetes mellitus, Type II (Non-Insulin-Dependent) diabetes, specific diabetes type like brittle
diabetes and gestational diabetes (Gutteridge 1999). The various forms of diabetes management include nutrition, physical activity, acupuncture and hydrotherapy, mineral supplementation and conventional therapy that include conventional drugs and transplantation (DeFronzo 1999). Conventional drugs include use of exogenous insulin and oral hypoglycaemic agents whose drawbacks are high cost, toxicity and other side effects. Because of these drawbacks, medicinal herbs have been used as alternative remedies to this disease.

1.2 Literature Review

1.2.1 Prevalence of diabetes mellitus

The prevalence of diabetes in adults globally was estimated to be 4.0% in 1995 and is projected to rise to 5.4% by the year 2025. It is projected that there will be a 4.2% increase in the number of individuals with diabetes, from 51 to 72 million in developed countries and 170% increase, from 84 to 228 million, in developing countries. The majority of people with diabetes in developing countries are projected to be younger, aged 45-64 years, while those in developed countries will be aged about 65 years. Diabetes will be increasingly concentrated in urban areas, with the greater burden of disease being among women (King et al. 1998).

In Sub Saharan Africa, it was estimated to be 0.01% in 2000 but projected to rise to 0.12% by the year 2025. In Kenya, it was estimated to be 1.06% in 2000 and would rise to 1.32% by the year 2025 (Hilary et al. 1998). Diabetes mellitus has affected several millions of people all over the world where it has a significant impact on the health, quality of life and life expectancy of patients, as well as on the health care systems (Jahodar 1993).
1.2.2 Role of Insulin in Metabolism and Diabetes Mellitus

The major function of insulin is to counter the concerted action of a number of hyperglycemia-generating hormones and to maintain low blood glucose levels. Because there are numerous hyperglycemic hormones, untreated disorders associated with insulin generally lead to severe hyperglycemia and shortened life span. In addition to its role in regulating glucose metabolism, insulin stimulates lipogenesis, diminishes lipolysis, and increases amino acid transport into cells. Insulin also modulates transcription, altering the cell content of numerous mRNAs. It stimulates growth, DNA synthesis, and cell replication, effects that it holds in common with the insulin-like growth factors (IGFs) and relaxin (Pirola et al. 2004).

Insulin is synthesized as a preprohormone in the β-cells of the islets of Langerhans. Its signal peptide is removed in the cisternae of the endoplasmic reticulum and it is packaged into secretory vesicles in the Golgi, folded to its native structure, and locked in this conformation by the formation of 2 disulfide bonds. Specific protease activity cleaves the center third of the molecule, which dissociates as C peptide, leaving the amino terminal B peptide disulfide bonded to the carboxy terminal A peptide (Pirola et al. 2004).

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 Ca²⁺ influx and insulin secretion. In fact, the role of K⁺ channels in insulin secretion presents a viable therapeutic target for treating hyperglycemia due to insulin insufficiency. Chronic increase in numerous other hormones, such as growth hormone, placental lactogen, estrogens, and progestins, up-regulate insulin secretion, probably by increasing the preproinsulin mRNA and enzymes involved in processing the increased preprohormone (Pirola et al. 2004; Robinson-White and Stratakis 2002).
Insulin, secreted by the β-cells of the pancreas, is directly infused via the portal vein to the liver, where it exerts profound metabolic effects. These effects are the response of the activation of the insulin receptor, which belongs to the class of cell surface receptors that exhibit intrinsic tyrosine kinase activity. The insulin receptor is a heterotetramer of 2 extracellular α-subunits disulfide bonded to 2 transmembrane β-subunits. With respect to hepatic glucose homeostasis, the effects of insulin receptor activation are specific phosphorylation events that lead to an increase in the storage of glucose with a concomitant decrease in hepatic glucose release to the circulation.

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).

In liver glucose uptake is dramatically increased because of increased activity of the enzymes glucokinase, phosphofructokinase-1 (PFK-1), and pyruvate kinase (PK), the key regulatory enzymes of glycolysis. The latter effects are induced by insulin-dependent activation of phosphodiesterase, with decreased PKA activity and diminished phosphorylation of pyruvate kinase and phosphofructokinase-2, PFK-2. Dephosphorylation of pyruvate kinase increases its' activity while dephosphorylation of PFK-2 renders it active as a kinase. The kinase activity of PFK-2 converts fructose-6-phosphate into fructose-2, 6-bisphosphate (F2,6BP). F2,6BP is a potent allosteric activator of the rate limiting enzyme of glycolysis, PFK-1, and an inhibitor of the gluconeogenic enzyme, fructose-1,6-
bisphosphatase (Pirola et al. 2004). In addition, phosphatases specific for the phosphorylated forms of the glycolytic enzymes increase in activity under the influence of insulin. All these events lead to conversion of the glycolytic enzymes to their active forms and consequently a significant increase in glycolysis. In addition, glucose-6-phosphatase activity is down regulated. The net effect is an increase in the content of hepatocyte glucose and its phosphorylated derivatives, with diminished blood glucose (Pirola et al. 2004).

In addition to the above described events, diminished cAMP and elevated phosphatase activity combine to convert glycogen phosphorylase to its inactive form and glycogen synthase to its active form, with the result that not only is glucose funneled to glycolytic products, but glycogen content is increased as well (Robinson-White and Stratakis 2002).

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) (Shepherd 2002). Activation of PI3K involves a linkage to receptor activation of insulin receptor substrates (of which there are four; IRS1, IRS2, IRS3 and IRS4). Activated PI3K phosphorylates membrane phospholipids, the major product being phosphotidylinositol 3, 4, 5 trisphosphate (PIP3). PIP3 in turn activates various enzymes, which include, the enzyme protein kinase B, PKB (also called Akt). The activated Akt is thought to initiate many of the metabolic actions of insulin (Shepherd 2002). PIP3 also activates enzymes such as PIP3-dependent kinase, PDK, some isoforms of protein kinase C, PKC (principally PKC-1) and small ribosomal subunit protein 6 kinase, S6K. The (Mitogen-activated protein kinases) MAP kinase pathway is also activated either through receptor activation of the protein tyrosine phosphatase (SHP-2) or growth factor receptor binding protein-2 (Grb2) (Shepherd 2002).

With respect to insulin responses, activation of PKB and PKC-1 lead to translocation of GLUT4 molecules to the cell surface resulting in increased glucose uptake, which is
significant in skeletal muscle. Activation of PKB also leads to the phosphorylation and inhibition of glycogen synthase kinase-3 (GSK3), which is a major regulatory kinase of glycogen homeostasis. In addition, PKB phosphorylates and inhibits the activity of a transcription factor (FKHRL1, now called FoxO3a) that has pro-apoptotic activity. This results in reduced apoptosis in response to insulin action (Shepherd 2002).

Activation of S6K leads to the phosphorylation of the regulator of translation eIF-4E binding protein, 4E-BP. Phosphorylation of 4E-BP prevents it from binding to eIF-4E the consequences of which would normally lead to a reduction in translation rate. 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 (but are not limited to) 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).

In contrast, epinephrine diminishes insulin secretion by a cAMP-coupled regulatory path. In addition, epinephrine 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 similarly to that of glucagon. The latter events induce glycogenolysis and gluconeogenesis, both of which are hyperglycemic and which thus counter insulin's effect on blood glucose levels. In addition, epinephrine influences glucose homeostasis through interaction with α-adrenergic receptors (Shepherd 2002).

Hormonal control of insulin action is also coupled to nutrient intake. Two of the many gastrointestinal hormones 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. GLP-1 is derived from the product of the proglucagon gene. This gene encodes a preproprotein that is differentially cleaved dependent upon the tissue in which it is synthesized. For example, in pancreatic α-cells prohormone convertase 2 actions leads to the release of glucagon. In the gut pro-hormone convertase $^{1/3}$ action leads to release of several peptides including GLP-1. Upon nutrient ingestion GLP-1 is secreted from intestinal entero-endocrine cells that are found predominantly in the ileum and colon with some production from these cell types in the duodenum and jejunum. Bioactive GLP-1 consists of 2 forms; GLP-1 (7-37) and GLP-1(7-36)amide, where the latter form constitutes the majority (80%) of the circulating hormone.

The primary physiological responses to GLP-1 are glucose-dependent insulin secretion, inhibition of glucagon secretion and inhibition of gastric acid secretion and gastric emptying. The latter effect will lead to increased satiety with reduced food intake along with a reduced desire to ingest food. The action of GLP-1 at the level of insulin and glucagon secretion results in significant reduction in circulating levels of glucose following nutrient intake. This activity has obvious significance in the context of this discussion of diabetes, in particular the hyperglycemia associated with poorly controlled type 2 diabetes. The glucose lowering activity of GLP-1 is highly transient as the half-life of this hormone in the circulation is less than 2 minutes. Removal of bioactive GLP-1 is a consequence of N-terminal proteolysis catalyzed by dipeptidyl peptidase IV (DPP IV). DPP IV is also known as the lymphocyte surface antigen CD26 and has numerous activities unrelated to incretin inactivation. All of the effects of GLP-1 are mediated following activation of the GLP-1 receptor (GLP-1R). The GLP-1R is a typical seven-transmembrane spanning receptor coupled to G-protein activation, increased cAMP production and activation of PKA.
However, there are also PKA-independent responses initiated through the GLP-1R. Other major responses to the actions of GLP-1 include pancreatic β-cell proliferation and expansion concomitant with a reduction of β-cell apoptosis (death). In addition, GLP-1 activity results in increased expression of the glucose transporter-2 (GLUT-2) and glucokinase genes in pancreatic cells (Robinson-White and Stratakis 2002). Therefore, insulin facilitates uptake of glucose, utilization and storage inform of glycogen.

Diabetes mellitus is a group of metabolic disorders with one common manifestation of hyperglycaemia due to lack of insulin. This also takes place due to the over-production of other hormones like glucagon, hormones of the anterior pituitary, adrenal and thyroid that are antagonists to insulin or due to increased production of insulinase, which inactivates insulin. Chronic hyperglycaemia causes damage to the eyes, kidneys, nerves, heart and blood vessels. The aetiology and pathophysiology leading to the hyperglycaemia, however, are markedly different among patients with diabetes mellitus, dictating different prevention strategies, diagnostic screening methods and treatments (Gutteridge 1999).

1.2.3 History of Diabetes Mellitus

Diabetes mellitus is one of the oldest known endocrine disorders. In India, Charaka and Sushruta described, “urine exceedingly sweet resembling the juice of sugar cane” which attracted ants about 2000 years ago. In China, Change-Ke is said to have been the first to describe sweet urine attracting dogs, possibly as early as the third century and certainly in the seventh century AD. The actual date remains uncertain since in some places it is as early as the sixth century BC, in others it was described in the first or second century BC. Wills was the first European physician to note in 1673 that the diabetic urine was wonderfully sweet as if it were imbued with honey or sugar (Mwanga 2000). It was until 1922 when insulin, the
first conventional drug, was discovered. Although very old and immensely investigated, diabetes mellitus remains a critical problem of public health today (Mwanga 2000).

1.2.4 Predisposing Factors

The major factors associated with the development of diabetes mellitus are:

1.2.4.1 Genetic Factors

The mode of inheritance of diabetes is complex. The transmission is polygenic. It is estimated that 30 to 50% of the offspring of two diabetic parents develop the disease (Mwanga 2000). Evidence for genetic predisposition also comes from studies on twins that show higher concordance rate for type 1 diabetes in monozygotic twins (25-30%) than in dizygotic twins (5-10%) (Skyler 1993).

1.2.4.2 Obesity and Nutrition

Obesity is the most important of acquired factors that contribute to the development of diabetes mellitus. Obesity predisposes a person to the development of diabetes related to the insulin resistance in the genetically predisposed individual with limited capacity of insulin secretion (Mwanga 2000).

Other causes of diabetes mellitus are destruction of islet cells resulting from the autoimmune response to viral infection, and also by receptor site defects that reduce the number of insulin-binding sites (Dods 1996).

1.2.5 Types of Diabetes

There are basically two major forms of diabetes mellitus: Insulin dependent diabetes mellitus (IDDM) also known as type I or juvenile diabetes and Non-insulin dependent
diabetes mellitus (NIDDM) also known as type II diabetes. Other forms are, gestational diabetes and secondary diabetes (like brittle diabetes) (Gutteridge 1999).

1.2.5.1 Type I Diabetes Mellitus

Type I diabetes mellitus is caused by failure to release insulin from the β-cells of the islets of Langerhans in the pancreas. Type I diabetes is also called Juvenile type because it often first manifests itself in the children and young adults. It is characterized by β-cell destruction caused by an autoimmune process, usually leading to absolute insulin deficiency. The onset is usually acute, developing over a period of a few days to weeks and the disease develops before the age of 25. Most of these patients have the "immune-mediated form" of type I diabetes mellitus with islet cell antibodies and often have other autoimmune disorders such as Hashimoto's thyroiditis, Addison's disease, and pernicious anaemia (National Diabetes Data Group 1979; Anonymous 1997).

Type 1 diabetes develops also due to a complex interaction between environmental factors and a genetic predisposition to the disease. Studies in monozygotic twins suggest that the genetic component accounts for 30-40% of the total risk (Tattersall and Pyke 1972; Hawkes 1997). Possible environmental triggers are exposure to viral infections such as rotavirus and different entero-viruses such as Coxsackie B Virus (Dahlquist 1994; Knip and Akerblom 1999).

1.2.5.1.1 Causes of Type I Diabetes Mellitus

1.2.5.1.1.1 Genetic Predisposition

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).

1.2.5.1.2 Environmental Factors

1.2.5.1.2.1 Nutrition

Nutrition given during the neonatal period and early infancy especially dietary factors such as neonatal exposure to Bovine serum antigen (BSA) in cow’s milk and chemical toxins in food and stress initiates injury to pancreatic β-cells (Dahlquist 1994; Knip and Akerblom 1999).

1.2.5.1.2.2 Exposure to Viruses and Allergens

An environmental insult, such as a virus, exposure to an allergen, or both, initiates the process in genetically susceptible individuals. This external influence precipitates an inflammatory response in the pancreas known as insulitis. Activated T-lymphocytes infiltrate the islet cells in the pancreas. Macrophages and T-cells lead to 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. The inflammatory response is autoimmune mediated and takes place on the surface of the insulin producing β-cells such that these cells are no longer recognized by the immune system. Antibodies against the β-cells are produced, resulting in their destruction. This destruction is thought to occur slowly, over the course of several years in many cases (Dahlquist 1994).

Some viruses seem to attack and destroy the β-cells directly. For example, exposure to enterovirus infections either in uterus or during childhood may initiate β-cell damage and subsequent type I diabetes (Dahlquist 1994).

1.2.5.1.1.2.3 Autoimmunity

Type 1 diabetes occurs in most cases due to autoimmune destruction of insulin producing pancreatic β-cells, leading to an absolute deficiency in insulin synthesis and secretion (Bach 1995). The autoimmune β-cell destruction may persist over a prolonged period prior to diagnosis of the disease, but loss of β-cell mass often accelerates markedly about 6 months before clinical presentations (Atkinson and Maclaren 1994). The autoimmunity can be anticipated to develop for several reasons. This is due to the presence of undetected infection where the immune response is directed entirely against an undetected pathogen, but it destroys the infected organ. For example, during hepatitis C and D infection which are associated with autoimmunity (Strassburg et al. 1996). Molecular mimicry may be involved because antigens against exogenous antigens can recognize certain antigens on the surface of the β-cell. In line with this, antibodies against Coxsackie B virus proteins cross-react with specific peptide sequences in β-cell proteins such as GAD. Uncontrolled cell death caused by non-pathogenic toxic agents or mutations in any of the many genes
controlling normal programmed cell death (apoptosis) or the scavenging process, could lead to immune cell activation. Thus the autoimmune response occurs because of their cellular distress. Also, a defective regulation of the immune response could shift the normal pattern of released antibodies and cytokines in the tissues. The β-cells are more sensitive to some cytokines and subsequently generate free radicals, than other cell types, and may therefore be damaged. Circulating autoantibodies against antigens such as GAD, insulin and uncharacterized cell-surface components are detectable in mostly newly diagnosed patients (Atkinson and Maclaren 1994; Leslie et al. 1999). These autoantibodies have been present for many years and they are also found in some subjects with apparently typical type 2 diabetes (Schranz and Lernmark 1998).

1.2.5.1.2.4 Cytokines

Infiltrating immune cells produce and release inflammatory mediators such as cytokines which may contribute to β-cell dysfunction and death (Mandrup-Poulsen 1996). Cytokines are small polypeptides and like hormones, they act as messengers between cells in order to control the immune system, inflammation, cell growth and haematopoiesis. Most cytokines act locally, but some such as IL-1β, IL-6 and TNF-α may also have systemic effects. A cytokine is often produced not only in immune cells but also in other cell types such as stroma cells and epithelial cells. Paradoxically, part of the β-cell destruction might be caused by cytokines produced by the β-cell themselves (Rabinovitch 1998). Several cytokines are expressed in type 1 diabetes and the pattern of the network in which these cytokines co-operate is very complex (Rabinovitch and Suarez-Pinzon 1998).
single signal. If no initial co-stimulation takes place, the T-cell will become anergic, that is unresponsive to any further stimulation by antigen (Ridge et al. 1998).

The principle is that these two signals activate but one may induce energy in an antigen specific response hence the cell provides a potential for targeted immunosuppressive therapy. Thus, activation of resting T-cells can be blocked by anti-B7 or anti-CD-154. The APCs can also be activated by unspecific endogenous alarm signals from distressed or damaged bodily tissues. These alarm signals may come in pre-packed or inducible form. The former might be any structure that is normally found inside but not outside the cell such as DNA, RNA, mitochondria and others. Inducible alarm signals may induce heat-shock proteins and some cytokines such as IL-1 and IFN-γ (Ridge et al. 1998).

1.2.5.1.2.7 Nitric Oxide (NO)

NO is the smallest known bioactive product of mammalian cells and can be produced by most cell types. It can be uncharged gas molecule, which has a high diffusion capacity and readily crosses cellular membranes. It has been identified as a cellular messenger putative neurotransmitter. Moreover, NO is a free radical that easily reacts with other molecules, especially those containing an unpaired electron, such as oxygen, iron, sulphur and nitrogen. For some enzymes, reaction with NO results in activation, whereas in others it inhibits the function of the protein. NO may also destroy iron-sulphur centre (4Fe/4S) within proteins important for the function of enzymes involved in the Krebs cycle, the mitochondrial respiratory chain and DNA synthesis/repair (Lowenstein 1994).

Nitric oxide synthase (NOS) catalyses the oxidation of arginine to citrulline and NO (Moncada et al. 1991). NOS exist in at least three different isomers; two are mostly constitutively expressed (cNOS), whereas one is inducible (iNOS) (Griffith and Stuehr 1995). The constitutive isoforms of NOS mediate endothelium-dependent relaxation (eNOS)
(Moncada, et al. 1991) and neural transmission (nNOS) and they are regulated by intracellular Ca\(^{2+}\) levels. eNOS is the most important endothelial derived relaxing factor in the body, and its presence is necessary to maintain a normal tissue blood flow. NO is produced in much larger amounts by iNOS, which is Ca\(^{2+}\) independent, and appears to mediate the cytotoxic actions of macrophages on target cells (Palmer et al. 1987).

Several of the cytokines induce iNOS production in different cell types, acting alone or in combination. In human pancreas islets, a combination of two (IL-1\(\beta\) + IFN-\(\gamma\)) or three (IL-1\(\beta\) + IFN-\(\gamma\) + TNF-\(\alpha\)) cytokines is required for iNOS activation. During certain conditions, cytokines may induce inhibitory actions in \(\beta\)-cells even when NO production is blocked. Therefore, the role of NO in human islets destruction is controversial and contradictory results exist (Ding et al. 1998).

1.2.5.1.2 Complications of type 1 diabetes

1.2.5.1.2.1 Metabolic Complications

Type 1 diabetes mellitus is characterized by increased urea and ketone body excretions in urine, lowered pH, increased water loss and increased thirstiness. Severe acidosis can lead to diabetic coma and if untreated can cause death (Mwanga 2000). Type 1 diabetes requires insulin injection to prevent ketosis, other complications and maintain life (Mwanga 2000).

1.2.5.1.2.2 Retinopathy

Cataract formation is the principal retinopathy of diabetes. Retinopathy is also caused by proliferation of small blood vessels in the lens due to hard exudates that are yellow, with irregular, sharply defined edges, varying in size from tiny specks to large confluent often
circular patches. These probably result from leakage of plasma from abnormal retinal capillaries and lie over areas of neuronal degeneration (Baird et al. 1986).

1.2.5.1.2.3 Neuropathy

It is apparent in about 25% of diabetics and is recognized by a variety of symptoms that include pain, numbness, tingling or burning sensations in extremities, dizziness and double vision. These symptoms are caused by decreased motor and sensory nerve conductional velocities caused by axonal degeneration and demyelination. Secondary manifestations of neuropathy include cardiac failure, excessive sweating and male impotence (Dods 1996).

1.2.5.1.2.4 Angiopathy

Angiopathy refers to damage to blood vessels of the lungs (basement membranes). Angiopathy increases the risk of coronary heart disease and stroke and can lead to retinopathy and nephropathy (Dods 1996).

1.2.5.1.2.5 Nephropathy

Capillary damage is caused by angiopathy. The result is a reduction in the filtering capability of the kidneys. Proteinuria is often the first sign of diabetic nephropathy. Approximately 25% to 30% of individuals treated for end-stage renal failure are diabetics (Dods 1996).
1.2.5.1.2.6 Infection

Diabetics are more prone to develop bacterial (mycobacterium and anaerobic) and fungal infections. This leads to ulceration and gangrene formation. Skin disorders are also more common in diabetics than in non-diabetics (Wheat 1980).

1.2.5.1.2.7 Diabetic Ketoacidosis (DKA)

In type I diabetics’ insulinopenia causes fat cells to mobilize fatty acids from triglycerides. Fatty acid degradation increases, as it becomes the major source of energy for the cell. Increased fatty acid metabolism produces excessive quantities of acetyl-CoA, which can enter tricarboxylic acid cycle to produce energy; an excess quantity of acetyl-CoA is metabolized to produce abnormal levels of keto acids (Ketosis). Increased production of keto acids consumes bicarbonate and thereby lowers blood pH (acidosis) (Dods 1996).

1.2.5.1.2.8 Diabetic Foot Disease

It is due to changes in blood vessels and nerves, often leads to ulceration and subsequent limb amputation. It is one of the most costly complications of diabetes, especially in persons with inadequate footwear. It results from both vascular and neurological disease processes. Diabetes is the most common cause of non-traumatic amputation of the lower limb (Wheat 1980).

1.2.5.1.2.9 Atherosclerosis

Atherosclerotic occlusive vascular disease is the most common complication of diabetes especially in type 2 diabetic patients. The lesions of atherosclerosis represent a series of highly specific cellular and molecular responses that can have many characteristics of an inflammatory disease. These lesions occur principally in large and medium sized
arteries and can lead to ischemia of the heart, brain, or extremities, resulting in infarction, stroke or peripheral extremity ischemia (Wheat 1980).

1.2.5.2 Type 2 Diabetes Mellitus

Type 2 diabetes mellitus is also known as non-insulin dependent diabetes mellitus (NIDDM) or maturity-onset type because it often first manifests itself in the adult, after 40 years of age especially if obese (Dods 1996). NIDDM represents a syndrome with disordered metabolism of carbohydrate and fat. The most prominent clinical feature is hyperglycaemia (fasting plasma glucose level > 126 mg/dL, or glycosylated haemoglobin Alc (Hb Alc) > 6.9%) (Anon 1997). NIDDM is characterized by insulin resistance in peripheral tissue and an insulin secretory defect of the β-cell (National Diabetes Data Group 1979; Anon 1997). The result is hyperinsulinism (excessive insulin), which may further depress receptors. The decrease in receptors may be body’s way of protecting itself from too much insulin (down regulation). Defective β-cells become exhausted, further fuelling the cycle of glucose intolerance and hyperglycaemia. The aetiology of type 2 diabetes mellitus is multifactorial and probably genetically based, but it also has strong behavioural components (National Diabetes Data Group 1979; Anon 1997).

This is the most common form of diabetes mellitus and is highly associated with a family history of diabetes, older age, obesity and lack of exercise. It is more common in women, especially women with a history of gestational diabetes (National Diabetes Data Group 1979). NIDDM is not dependent on insulin injection and accounts for over 90% of all diabetes cases worldwide (WHO 1985; 1994).
1.2.5.2.1 Causes of type 2 Diabetes

1.2.5.2.1.1 Genetic Factors

Type 2 diabetes mellitus shows strong familial aggression. Twins and family studies have shown firm evidence that the role of the genetic component is relatively strong. Several genes have been suggested as markers for type 2 but apart from evidence for abnormalities in the adenosine deaminase and glucokinase genes, no other consistent abnormalities have yet been found (Frognel 1992).

1.2.5.2.1.2 Environmental Factors

Increased intake of saturated fats and decreased intake of dietary fibre can result in a decrease of insulin sensitivity and abnormal glucose tolerance. This diet is also accompanied by other changes such as arterial hypertension, dislipidaemia and obesity. Severe or prolonged stress/trauma are associated with glucose intolerance induced by hormonal effects on glucose metabolism and insulin secretion and action. However, this remains unproven (Frognel 1992).

Drugs also impair glucose metabolism. These include phenytoin, diuretics (particularly of the thiazide type), corticosteroids, some contraceptive steroids and β-adrenoreceptor antagonists agents, which may cause glucose intolerance and in susceptible individuals, may induce diabetes. This resolves after withdrawal of the drug (Frognel 1992).

1.2.5.2.2 Complications of type 2 Diabetes

The quality of life of type 2 patients with chronic and severe hypoglycaemia is adversely affected. It is characterized by tiredness and lethargy and can become severe and lead to a decrease in work performance in adults and increased fall in the elderly (Davidson 1991).
Acute complications include metabolic problems (hyperosmolar hyperglycaemia non-ketotic syndrome or HHNS) and infection. The long-term complications are macrovascular complications (hypertension, dyslipidemia, myocardial infarction, stroke), micromascular complications (retinopathy, nephropathy, diabetic neuropathy, diarrhoea, impaired cardiovascular reflexes, sexual dysfunction), and diabetic foot disorders (Davidson 1991).

The hypovolemia associated with these acute illnesses can result in shock and renal failure. Loss of salts usually occurs in diabetic ketoacidosis (DKA) and hyperosmolar hyperglycaemia non-ketotic syndrome (HHNC). Although patients’ serum electrolytes may be elevated, normal or low, they usually have a deficit of body potassium (Davidson 1991).

1.2.5.2.2.1 Specific Diabetes-related Micro vascular Complications

1.2.5.2.2.1.1 Retinopathy and Nephropathy

These relate predominantly to small vessel disease, characterized by progressive obliteration of capillaries. This occurs throughout the body, but mainly in the kidney and in the eyes. The capillaries in the renal glomerulus and in the retina are “end capillaries” with little communication to each other, so that when one capillary closes the tissue perfused by that capillary becomes ischaemic. The cause of capillary closure is uncertain, but it seems possible that the pericyte cells, which help to maintain the integrity of the endothelial cells lining the capillaries, are susceptible to hyperglycaemia. One hypothesis is that these cells have enhanced sugar metabolism via the aldose reductase leading to sorbitol accumulation and consequent metabolic disturbances. Increased platelet stickiness is also involved and increased glycosylation of several different proteins may be a factor. The basement membrane of the capillaries becomes thickened, particularly in the mesangium, the central part of the renal glomerulus (Davidson 1991).
In the kidneys, a nephritic syndrome occurs when damaged basement membrane of the glomerulus allows excess filtration of albumin. In the retina, fragile vessels in response to ischemia occur. These bleed to form an intra-ocular, vitreous haemorrhage which causes sudden loss of vision. Ischemia of the retina can also lead to direct blindness when it affects the macula, which is the most sensitive central part of the retina (Davidson 1991).

### 1.2.5.2.1.2 Polyneuropathy

Diabetes also leads to a severe polyneuropathy. This is characterized by the demyelination of segments of many nerves. This leads to peripheral loss of sensation and motor weakness. The inability to sense trauma and abnormal pressure on the foot due to muscle weakness lead to foot ulcers and infection. Polyneuropathy also leads to impotence (Davidson 1991).

The development of all these types of complications is due to increased glycosylation of proteins. This lead to advanced glycosylation end-products (AGE), which induce cross-linking of proteins or macrophage attack with local release of lymphokines, which cause cellular damage (Davidson 1991).

### 1.2.5.3 Secondary Diabetes

Diabetes mellitus caused by other conditions and diseases is known as secondary diabetes. This occurs as a result of damage to the pancreas due to chronic pancreatitis and pancreatic carcinoma. Secondary diabetes also occurs due to the presence of insulin antagonists such as, excess growth hormone (acromegaly), elevated cortisol (Cushing’s syndrome), excessive catecholamines (pherochromocytoma), excessive glucagon because of a tumour (glucagonoma), excessive somatostatin because of a tumor (somatostatinoma), primary aldosteronism, severe liver disease, and administration of certain drugs, hormones
and chemicals. Inhibition of insulin secretion from the β-cells by the excess secretion of epinephrine and thyroxine resulting to breakdown of liver glycogen also leads to secondary diabetes (Dods 1996).

1.2.5.4 Gestational Diabetes

Gestational diabetes mellitus (GDM) defined as glucose intolerance detected during pregnancy, occurs in approximately 2-5% of pregnancies and is considered to be one of the common complications for pregnancy. This diabetic condition develops during the second or third trimester of pregnancy. Women with GDM are at risk of developing diabetes and their offspring may be at increased risk of developing obesity (WHO 1985).

Gestational diabetes is more common in women who have had several pregnancies. Pregnancy is characterized by increased insulin resistance from placental lactogen secretion hence pregnancy exacerbates the effect of insulin resistance so inducing the disease. The condition is usually treated by diet; however, insulin injections may also be required (WHO 1985).

1.2.6 Diagnosis of Diabetes Mellitus

The new diagnostic criteria for diabetes mellitus have been greatly simplified. The oral glucose tolerance test previously recommended by the National Diabetes Data Group has been replaced with the recommendation that the diagnosis of diabetes mellitus be based on two fasting plasma glucose levels of 7.0 mmol/L or higher. Other options for diagnosis include two two-hour postprandial plasma glucose (2hrPPG) readings of 11.1 mmol/L or higher after a glucose load of 75 g (essentially, the criteria recommended by WHO) or two casual glucose readings of 11.1 mmol/L or higher. Measurement of the fasting plasma glucose level is the preferred diagnostic test, but any combination of two abnormal test
results can be used. Fasting plasma glucose was selected as the primary diagnostic test because it predicts adverse outcomes (like retinopathy) as well as the 2hr PPG test but is much more reproducible than the oral glucose tolerance test or the 2hr PPG test and easier to perform in a clinical setting (WHO 1985; Gutteridge 1999).

The choice of the new cut off point for fasting plasma glucose levels is based on strong evidence from a number of populations linking the risk of various complications to the glycaemic status of the patient. The risk of retinopathy greatly increases when the patient's fasting plasma glucose level is higher than 6.05 to 6.45 mmol/L or when the result of a 2hr PPG test is higher than 8.3 to 10.0 mmol/L. However, the committee decided to maintain the cut-off point for the 2hr PPG test at 11.1 mmol/L because so much literature has already been published using this criterion. They selected a cut off point for fasting plasma glucose of 7.0 mmol/L or higher. This point corresponded best with the 2hr PPG level of 11.1 mmol/L. The risk of other complications also increases dramatically at the same cut off points (WHO 1985; Gutteridge 1999).

A normal fasting plasma glucose level is less than 6.1 mmol/L and normal 2hr PPG levels are less than 7.75 mmol/L. Blood glucose levels above the normal level but below the criterion established for diabetes mellitus indicate impaired glucose homeostasis. Persons with fasting plasma glucose levels ranging from 6.1 to 7.0 mmol/L are said to have impaired fasting glucose, while those with a 2hr PPG level between 7.75 mmol/L and 11.1 mmol/L are said to have impaired glucose tolerance. Both impaired fasting glucose and impaired glucose tolerance, the committee chose not to address the current controversies surrounding the diagnosis of gestational diabetes mellitus and did not alter the diagnostic criteria in this area. Screening for gestational diabetes mellitus is generally accomplished with administration of a 50 g glucose load one hour before determining a plasma glucose level. A positive screen (defined as a plasma glucose level of 7.75 mmol/L or higher) should prompt a diagnostic test:
fasting plasma glucose levels should be measured after a 100 g glucose load at baseline and at one, two and three hours after the glucose load. Two of the four values must be abnormal (5.8 mmol/L or higher; 10.5 mmol/L or higher; 9.15 mmol/L or higher and 8.05 mmol/L or higher for a patient to be diagnosed with gestational diabetes mellitus). Diabetes mellitus is confirmed by fasting plasma glucose ≥ 7 mmol/L, with a reduced reliance on the role of oral glucose tolerance testing (WHO 1985; Gutteridge 1999).

1.2.7 Management of Diabetes Mellitus

1.2.7.1 Diet Therapy

Given the heterogeneous nature of type 2 diabetes, no single dietary approach is appropriate for all patients. Meal plans and diet modifications are generally individualized by a registered dietician to meet patient needs and lifestyle. A typical conventional approach would recommend a diet composed of 60-65% carbohydrate, 25-35% fat, and 10-20% protein with limited or no alcohol consumption (Schilichtmann and Graber 1997). The diabetic diet is designed to control both the disease and its complications and meet nutritional needs (Mwanga 2000).

The number of calories required depends on the patient's age, weight, and activity level. The calorie intake also needs to be distributed over the course of the entire day so surges of glucose entering the blood system are kept to a minimum. Keeping track of the number of calories provided by different foods can be complicated, so patients are usually advised to consult a nutritionist or dietician (Schilichtmann and Graber 1997).

1.2.7.2 Vegetables

Vegetables are among the numerous plant adjuncts tried on the treatment of diabetes mellitus. Bitter gourd (Momordica charantia) and Ivy gourd (Coccinia indica) are
hypoglycaemic when administered orally. Other vegetables such as cabbage (*Brassicia oleracia*) green leafy vegetables, beans and tubers are hypoglycaemic in both experimental animals and humans (Platel and Srinivasan 1997).

1.2.7.3 Mineral Supplementation

The treatment of diabetes requires nutritional supplementation, as these patients have a greatly increased need for many nutrients. Supplying the diabetic with additional key nutrients improves blood sugar control as well as help prevent many major complications of diabetes.

1.2.7.3.1 Chromium

Chromium (Cr) is an essential element required for normal lipid and carbohydrate metabolism. It is not formed by the human body and therefore needs to be supplemented (Mertz 1975). It is absorbed by the roots of plants, including grains, and finds its way into the food chain as a biologically active organic complex, known as Glucose-Tolerance-Factor (GTF), as yet chemically unidentified chromium-containing compound, which is readily absorbed in the body. GTF has been shown to act as a cofactor with zinc for insulin utilization in glucose metabolism (Morris *et al.* 1992). It is non-toxic, and is more readily metabolized than the inorganic salts of chromium, which are very poorly absorbed by the gastrointestinal tract and may cause some irritation (Mertz 1975).

The estimated total chromium in an average adult is less than 6 mg. It is widely distributed in human tissues in extremely low concentrations, 0.02 to 0.04 µg/g, on a dry-weight basis. The concentration of chromium in the human pancreas range between 0.10 to 0.85 µg/g. The chromium concentration in organs from diabetic subjects is significantly lower than that obtained from non-diabetic (Schroeder 1968).
The role of chromium in the body is in glucose and lipid metabolism, and a dietary deficiency can cause impaired glucose tolerance, which is of great importance to diabetics. Oral supplementation with chromium corrects this problem in patients with NIDDM and in children with protein-energy malnutrition, but such supplements have no effect in people with normal chromium intakes.

Chromium that is an essential micronutrient for humans functions as a cofactor in insulin-regulating activities. It facilitates insulin binding and subsequent uptake of glucose into the cell and therefore decreases fasting glucose levels, improves glucose tolerance, lowers insulin levels and decreases total cholesterol in normal, elderly, and type 2 diabetic subjects (Mooradian et al. 1994; Baker 1996). Without chromium, insulin’s action is blocked and glucose levels are elevated (Mooradian et al. 1994). Chromium picolinate is one of the forms of chromium that exhibits biological activity (Mertz 1969). Trivalent chromium has long been considered to be a safe nutritional supplement (Castro 1998). Although the hexavalent form of chromium is a known human respiratory tract carcinogen when inhaled in high-exposure industrial settings, there is no evidence of any carcinogenic effects in humans from the trivalent form of chromium found in chromium supplements. A reasonable amount of supplemental chromium is 200 μg/day (Castro 1998).

Brewers yeast appears to be the richest source of GTF-chromium, followed closely by black pepper, wheat germ, rye bread, mushrooms, prunes, wine, and beer. Most meats, fresh fruits, and cheeses are fair sources of chromium. Cereals are poorer sources, their chromium decreasing with refining and processing (Castro 1998).

1.2.7.3.2 Vanadium

Vanadium (V) compounds act in an insulin-mimetic fashion both in vitro and in vivo. Both inorganic and organic vanadium compounds have been shown to lower plasma glucose
levels, increase peripheral glucose uptake, improve insulin sensitivity, decrease plasma lipid levels and normalize liver enzyme activities in a variety of animal models of both type I and type II diabetes. Vanadium treatment of diabetic animals does not restore plasma insulin levels but may spare pancreatic insulin (Orvig et al. 2000). Vanadium is also known to play a role in the regulation of intracellular signalling and as a cofactor of enzymes essential in energy metabolism hence reduces gluconeogenesis and increases glycogen deposition. A reasonable amount of supplemental vanadium is 20 µg/day. Vanadyl sulfate at a dose of 100 mg/day is effective in improving insulin sensitivity. Good sources of vanadium include seafood, mushrooms, olives, whole grain breads, carrots and vegetable oils (Cohen et al. 1995; Halberstam et al. 1996).

1.2.7.3.3 Magnesium

Magnesium (Mg) is one of the major mineral constituents of human body. Its functions include strengthening cell membrane structure, cofactor to several enzymes like kinase, which participate in energy production processes and participation in deoxyribonucleic acid (DNA) replication (McNair et al. 1978).

A deficiency of magnesium is significantly more common in type 2 diabetics than in the general population (Sjogren et al. 1988). Magnesium deficiency has been associated with complications of diabetes, retinopathy in particular. One study found patients with severe retinopathy had reduced levels of magnesium. However, excess magnesium impairs kidney function. A reasonable amount of supplemental magnesium is 450 mg/day (McNair et al. 1978).
1.2.7.3.4 Zinc

Zinc is an important trace element in diabetes. It is a cofactor for insulin. Although its real mechanisms in carbohydrate metabolism is not clear, Zinc has influence in carbonic anhydrase, alkaline phosphatase, alcohol dehydrogenase, pancreatic carboxypeptidases A and B, lactate dehydrogenase, glutamate dehydrogenase, glyceraldehyde-3-phosphate dehydrogenase and maltose dehydrogenase. Zinc plays a vital role in the biosynthesis of nucleic acids, RNA polymerases, and DNA polymerases; hence its involvement in the healing processes of body tissues. Other physiological processes that require zinc include hormone metabolism, immune responses and stabilization of ribosomes and membranes (Ouma 1994).

1.2.7.3.5 Manganese

Manganese (Mn) is a trace metal in the body. It is both an activator and a constituent of several enzymes. It is necessary for normal activity of hydrolyases, kinases, decarboxylases, transferases, leucine amino peptidase, alkaline phosphatase and of the enzymes of oxidative phosphorylation. Manganese metalloenzymes include pyruvate decarboxylase, arginase, glutamate synthetase, and manganese superoxide dismutase (Friedman 1987).

1.2.7.3.6 Molybdenum

Molybdenum (Mo) affects glucose metabolism. In the hepatocytes, molybdenum stimulates glycolysis and accelerates glycogen degradation (Fillat et al. 1992). Mo also increases insulin receptor autophosphorylation and phosphorylation of its substrate and augments glucose transport, oxidation and lipogenesis in adipocytes (Goto et al. 1992; Li et al. 1995).
Molybdate is an effective antihyperglycemic agent in diabetics with severe insulin resistance. It is associated with a substantial reduction of hyperinsulinaemia and an increase in pancreatic insulin stores. The glucose-lowering effect of Mo may be partly related to attenuation of hepatic glucose production (and possibly also to increased glucose usage). Hence, Mo proves to be an effective blood glucose-lowering agent in severely diabetic patients (Reul et al. 1997).

1.2.7.3.7 Iron

Iron is an important element in the transportation of oxygen from the lungs by way of the blood stream to the tissues. It is present in the red blood cell protein, haemoglobin. A similar protein in muscle, myoglobin, also contains iron and stores oxygen for use during muscle contraction. Iron is found in the portion of the cell involved in energy production and as a cofactor for several enzymes like succinic dehydrogenase, catalase, and cytochromes. Studies show that there is a relationship between iron metabolism and type 2 diabetes. The relationship is bi-directional; iron affects glucose metabolism and glucose metabolism impinges on several iron metabolic pathways. Insulin as an anabolic hormone stimulates the cellular uptake of many nutrients, including hexoses, amino acids, cations and anions. Intestinal absorption of non-heme iron is tightly regulated in keeping with the body requirements, and absorption of iron is minimal when body iron stores are normal. Absorption of heme iron does not appear to be dependent on body iron content (Jose' Manuel et al. 2002). Insulin is known to cause a rapid and marked stimulation of iron uptake by fat cells, redistributing transferrin receptors from an intracellular membrane compartment to the cell surface. Insulin is also responsible for the increased ferritin synthesis (Yokomori et al. 1991). Reciprocally, iron influences insulin action. Iron interferes with insulin inhibition of glucose production by the liver. Hepatic excretion and metabolism of insulin is reduced with
increasing iron stores, leading to peripheral hyperinsulinaemia (Niederau et al. 1984). In fact, the initial and most common abnormality seen in iron overload conditions is liver insulin resistance (Dandona et al. 1983). Iron overload also affects skeletal muscle, the main effector of insulin action (Shafer et al. 1981).

Oxidative stress and inflammatory cytokines influence these relationships, amplifying and potentiating the initiated events. Oxidative stress induces both insulin resistance by decreasing internalization of insulin and increasing ferritin synthesis (Bertelsen et al. 2001). Iron is intimately linked to oxidative stress. Iron participates, through the Fenton reaction, in the formation of highly toxic free radicals, such as hydroxide and the superoxide anion, which are capable of inducing lipid peroxidation. For iron to act as a pro-oxidant agent, it must be in its free form. Iron can be released from ferritin by the action of reducing agents that convert Fe$^{3+}$ into Fe$^{2+}$. Glycation of transferrin decreases its ability to bind ferrous iron and, by increasing the pool of free iron, stimulates ferritin synthesis. Glycated holotransferrin is additionally known to facilitate the production of free oxygen radicals, such as hydroxide, that further amplify the oxidative effects of iron. Cytokines simultaneously cause an increase in transferring receptors on the cell surface, favoring tissue deposition of iron and insulin resistance (Juckett et al. 1995). The clinical impact of these interactions depends on both the genetic predisposition and the time frame in which this network of closely related signals acts. Increased iron stores predict the development of type 2 diabetes while iron depletion is protective.

Iron-induced damage might also modulate the development of chronic diabetes complications. The general effect of catalytic iron is to convert poorly reactive free radicals, such as H$_2$O$_2$, into highly reactive ones, such as OH$^-$ and O$_2^\cdot$. Free radicals and other oxidation by-products impair the mechanisms of vasodilatation and cause endothelial depletion of endogenous antioxidants, such as ascorbic acid. Iron chelation blocks oxidation
of low-density lipoprotein (LDL), and iron released from heme and ferritin favors oxidation of this lipoprotein (Abdalla et al. 1992). Increased iron availability contributes to macrovascular disease because iron has an adverse effect on endothelium and accelerates the development of atherosclerosis. Ferritin gene expression increases in the course of atherosclerotic plaque formation (Abdalla et al. 1992). Iron depletion is beneficial in coronary artery responses, endothelial dysfunction, insulin secretion, insulin action, and metabolic control in type 2 diabetes (Jose' Manuel et al. 2002).

1.2.7.4 Physical Activity

In well-controlled diabetes, physical activity improves the body's ability to use glucose and lowers the insulin requirement (Mwanga 2000). Exercise should start at a low level and gradually increase to avoid adverse effects such as injury, hypoglycaemia, or cardiac problems (Alexandria 1994; American Diabetes Association 1997).

1.2.7.5 Acupuncture and Hydrotherapy

1.2.7.5.1 Acupuncture

Acupuncture is the best-known alternative therapy in the United States of America for chronic pain and is used in the treatment of diabetes. Acupuncture is effective in treating not only diabetes, but also in preventing and managing complications of the diseases (Hui 1995). Acupuncture activates glucose-6-phosphatase an important enzyme in carbohydrate metabolism and affects the hypothalamus. Acupuncture acts on the pancreas to enhance insulin synthesis, increase the number of receptors on target cells, and accelerate the utilization of glucose, resulting in lowering of blood sugar. Acupuncture has also an anti-obesity effect, which is the most modifiable risk factor for type 2 diabetes. The therapeutic
The effect of acupuncture on diabetes is not the result of its action on one single organ, but on multiple systems (Hui 1995).

The four commonly used points are; Zusanli point located three inches below the lateral knee depression, one finger width from the lateral side of the anterior crest of the tibia, Sanyinjiao point which is located three inches above the tip of the inner ankle, on the posterior margin of the metatarsal bone, Feishu point located 1.5 inches lateral and inferior to the spinous process of the third thoracic vertebra in a prone position and Shenshu point, which is located 1.5 inches lateral to the posterior midline, lateral and inferior to the spinous process of the second lumbar vertebra in a prone position (Hui 1995).

The acupuncture points are selected based on traditional Chinese medicine theory. During treatment, other points can be added according to symptoms and signs (Hui 1995). Other methods have also been employed such as point injection with normal saline, small dose insulin, and Chinese herbal medicine extracts.

Acupuncture can be effective in treating complications of diabetes and is promising in patients with dietary control, physical exercise, breathing exercises and massage. Although acupuncture shows some effectiveness in treating diabetes, its mechanisms are still obscure (Hui 1995).

1.2.7.5.2 Hydrotherapy

Since hot-tub therapy can increase blood flow to skeletal muscles, it has been recommended for patients with type 2 diabetes who are unable to exercise. Hot-tub therapy decreases weight, mean plasma glucose level, and mean glycosylated haemoglobin (Hooper 1999). However, caution should be taken that the water is not too hot as neuropathy may prevent the patient from noticing that they are burning themselves; proper water sanitation and appropriate guidance should be considered (Hooper 2000).
1.2.7.6 Conventional Management

1.2.7.6.1 Insulin Therapy (Exogenous Insulin)

Insulin therapy restores normoglycemia, suppressing ketogenesis, delaying or arresting diabetic complications (Garg 2002). The insulin is given only by injections (Mwanga, 2000). Insulin also stimulates the synthesis of glucokinase and moderates the degree of gluconeogenesis (Johadar 1993).

Weight gain, hypoglycaemia, skin reactions, insulin resistance due to antibody reaction, insulin lipidystrophy, visual disturbance and allergy are common side effects of insulin therapy (UKPDS 1998a, b, c; Sinha et al. 1996). Vigorous insulin treatment also carries an increased risk of atherogenesis (UKPDS 1998c). Insulin therapy is also unavailable to many communities in developing countries due to inaccessible health facilities and socio-economic factors (WHO 1996).

1.2.7.6.2 Oral glucose – lowering agents

1.2.7.6.2.1 Sulfonylurea

These include sulfonylurea such as tolbutamide and glyburide. 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). This enhances the glycolytic flux and inhibits glucose output from the liver inhibiting gluconeogenesis (Parving et al. 1992; Kelly 1995). Thus, these drugs could be used only in patients with type 2 diabetes having functional beta cells for endogenous insulin production. Other contributory mechanisms for these compounds have also been suggested, such as, involvement of protein kinase C, increase in cAMP level and calcium ionophore-like activity (Chakrabarti and Rajagopalan 2002).
With the trademark labelling of tolbutamide (Orinase) by the United States of America Food and Drug Administration in 1962, the sulfonylurea class of drugs quickly became the mainstay of treatment for type 2 diabetes. Although newer agents have recently entered the marketplace, sulfonylureas still play a primary role in pharmacologic management of type 2 diabetes. Patients who respond best to treatment with sulfonylureas include those with a diagnosis of type 2 diabetes below 40 years of age, duration of disease less than five years before initiation of drug therapy and a fasting blood glucose level of less than 16.7 mmol/L (Mooradian 1996).

Approximately two thirds of patients who begin therapy with a sulfonylurea respond, although up to 20 percent of them eventually require additional medication. Few patients with uncontrolled diabetes receive clinical benefit when switched from one sulfonylurea agent to another (Mooradian 1996). The use of agents with a longer half-life such as chlorpropamide (Diabinese) in the elderly and in patients with renal impairment is discouraged because the risk of hypoglycaemia is increased.

A significant side effect is hypoglycaemia and weight gain due to hyperinsulinaemia (Parving et al. 1992; Kelly 1995). The weight gain is implicated as a cause of secondary drug failure (De Fronzo 1999; Parving et al. 1992; Kelly 1995).

1.2.7.6.2.2 Biguanides

These reduce hepatic glucose output, fasting glucose output and fasting glucose levels by increasing hepatic insulin sensitivity. They reduce intestinal absorption of glucose (Mwanga 2000). These include the drug metformin derived from a medicinal plant, Galega officinalis (DeFronzo 1999). Metformin is a biguanide agent that lowers blood glucose primarily by decreasing hepatic glucose production, and increases muscle glucose uptake. It also reduces plasma triglyceride and LDL-cholesterol levels and reducing insulin resistance.
Metformin is used as monotherapy or in combination with sulfonylureas for management of type 2 diabetes. When used as monotherapy, metformin does not cause hypoglycaemia and is thus termed an “antihyperglycemic.” The use of metformin is contraindicated in patients with renal insufficiency (that is, a serum creatinine level of 130 µmol/L in men and 120 µmol/L in women, or abnormal creatinine clearance) or acute or chronic metabolic acidosis. Metformin should be temporarily withheld before any procedure involving intravascular administration of iodinated contrast media. Normal renal function should be confirmed 48 hours after the procedure before restarting metformin therapy. Extreme caution is taken in patients with severe hepatic dysfunction, hypoxemic states (such as severe chronic obstructive pulmonary disease and congestive heart failure), and moderate to severe illness and excessive alcohol intake. In these patients, the use of metformin may contribute to the development of lactic acidosis, a condition that is fatal in about 50 percent of patients who develop it (Scheen 1997). Cimetidine (Tagamet) decreases the renal clearance of metformin and may potentiate its effects. Patients receiving oral anticoagulant therapy and metformin may require a higher dosage of warfarin (Coumadin) to achieve a therapeutic anti-thrombotic effect (Melchior 1996). Haemoglobin, haematocrit, red blood cell indexes and renal function should be monitored at least annually in patients taking metformin.

The side effects include weakness, fatigue, shortness of breath, nausea, dizziness, lactic acidosis and kidney toxicity (DeFronzo 1999).

1.2.7.6.2.3 Alpha-glucosidase inhibitors

Alpha-glucosidase inhibitors, such as acarbose (Precose) and miglitol (Glyset), are indicated as monotherapy or in combination with sulfonylureas for management of type 2 diabetes. These 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). Acarbose and miglitol should be titrated over two to three weeks to minimize flatulence and other gastrointestinal side effects that commonly lead to discontinuation of these agents. \(\alpha\)-glucosidase inhibitors are contraindicated in patients with inflammatory bowel disease, partial intestinal obstruction, and a predisposition to intestinal obstruction, colonic ulceration and other gastrointestinal disorders (Campbell et al. 1996).

Dose-dependent hepatotoxicity is associated with this drug class, so liver function tests should be carefully monitored in patients receiving higher dosages of these medications. Transaminase elevations are reversible with discontinuation of the drug and are often asymptomatic. Serum transaminase levels should be checked every three months for the first year patients taking the medication, and periodically thereafter. Drugs that are susceptible to binding with other agents (cholestyramine) should be taken two to four hours apart from \(\alpha\)-glucosidase inhibitors to avoid drug interactions. Intestinal absorbents and digestive enzyme preparations should not be administered with acarbose. The major side effects are gas, bloating and diarrhoea (Raing et al. 2000).

1.2.7.6.2.4 Thiazolidinediones

These are represented by troglitazone, rosiglitazone and pioglitazone. The thiazolidinediones are a unique drug class of "insulin sensitizers" that promote skeletal muscle glucose uptake and to a much lesser extent, in the liver (Sparano and Seaton 1998). Troglitazone is the first agent of this drug class to be introduced in the United States of America market and, like metformin, it reduces insulin resistance. Troglitazone is beneficial in patients requiring large daily amounts of insulin (more than 30 units per day) whose diabetes is still uncontrolled. A reduction of up to 50 % in total daily insulin dosage is possible with drug titration. Troglitazone is also effective when used in combination with
other oral agents thereby potentially delaying the need to start insulin therapy (Inzucchi et al. 1998).

Molecular mechanisms of action of these agents are through binding avidly to peroxisome proliferator-activated receptor gamma (PPARγ). PPARγ is a member of a family of nuclear receptors. Another member of this class, peroxisome proliferators activated receptor alpha (PPARα), is predominantly expressed in the liver and mediates the triglyceride lowering actions of fibrates. PPARγ is expressed in many tissues, including colon, skeletal muscle, liver, heart and activated macrophages, but is most abundant in adipocytes (Greenfield and Chisholm 2004).

Thiazolidinediones are selective agonists of PPARγ. When activated by a ligand, such as a thiazolidinedione, PPARγ 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 PPARγ 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 PPARγ stimulation are not seen in visceral adipocytes, even though these cells have abundant PPARγ receptors (Greenfield and Chisholm 2004). Visceral adipocytes are also metabolically quite different to peripheral adipocytes like being less responsive to insulin and more responsive to catecholamines. Increased fatty acid storage in subcutaneous adipocytes results in a 'lipid-steal' phenomenon, leading to lower circulating fatty acids and reduced concentrations of triglycerides in muscle and liver.

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 PPARγ stimulation in muscle and liver are unclear (Greenfield and Chisholm
2004).

The potential role of the thiazolidinediones in reducing hepatic lipid content in non-
alcoholic steatohepatitis is still under investigation. The thiazolidinediones do not increase
insulin secretion. On the contrary, thiazolidinediones reduce insulin levels acutely, which
may be a consequence of improved insulin sensitivity and/or reduced circulating fatty acids
(as fatty acids stimulate insulin secretion). In the longer term, thiazolidinediones arrest the
decline in β-cell function that occurs in type 2 diabetes, perhaps by protecting the β-cell from
lipotoxicity. The thiazolidinediones are of no use in type 1 diabetes or in the occasional lean
insulin-deficient (but insulin-sensitive) patient with type 2 diabetes (Greenfield and Chisholm
2004).

In addition to promoting adipogenesis and fatty acid uptake, 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, again via PPARγ activation, substantially 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).
There has been an interesting discussion about the degree to which the improved insulin response induced by thiazolidinediones is mediated by increased glucose processing molecules (such as the insulin regulated glucose transporter, GLUT 4, and pyruvate dehydrogenase activity) in adipocytes (Lee et al. 2003). As adipocytes only account for a small component of insulin-induced glucose disposal, it seems likely that the effects of thiazolidinediones on these glucose handling proteins are not a major component of their activity (Lee et al. 2003).

The disadvantage of these drugs is that they are expensive oral agents. These drugs decrease plasma triglyceride levels, but such decrease may be associated with weight gain and an increase in LDL – cholesterol levels (Raing et al. 2000). Hepatotoxicity is a concern requiring monthly monitoring of liver function every month for the first eight months of treatment and every other month for four months thereafter (Sparano and Seaton 1998).

1.2.7.6.2.5 Meglitinides

One of the meglitinides is repaglinide. Repaglinide is an insulin secretagogue, the first of the meglitinide class. It is a member of the carbamoyl methyl benzoic acid family (glinides) introduced in early 1998. It is structurally different from the traditional sulfonylureas, but shows chemical resemblance to the nonsulfonylurea moiety of the glibenclamide molecule (Chakrabarti and Rajagopalan 2002).

Nateglinide, the newest member of the class has recently become available. The meglitinides stimulate the release of insulin from the pancreatic β-cells. However, this action is mediated through a different binding site on the ‘sulfonylurea receptor’ of the β-cells and the drugs have somewhat different characteristics when compared with sulfonylureas. In contrast to glibenclamide, meglitinides do not stimulate calcium dependent exocytosis (Chakrabarti and Rajagopalan 2002).
Glibenclamide, not meglitinide, can stimulate insulin secretion \textit{in vitro} even in the complete absence of glucose, whereas in presence of 5 or 10 mmol/l of glucose, meglitinides are 5 times more potent than glibenclamide in insulin secretion (Fuhlendorff \textit{et al.} 1998). Unlike commonly used sulfonylureas, the meglitinides have a very quick onset of action and a short half-life. Some potential advantages of this class of agents include a greater decrease in post-prandial glucose and a decreased risk of hypoglycemia. Because of their quick onset of action (15 to 30 min), patients should be instructed to administer a dose immediately before a meal. If a meal is omitted throughout the day, patients should be instructed to skip the corresponding dose to prevent hypoglycemia. Likewise, if an extra meal is added throughout the day, the patient should add a dose to cover that meal. Thus, unique dosing regimen may allow greater flexibility for patients who have difficulty in maintaining a regular meal schedule. Besides the two above-mentioned drugs, Servier has a molecule (Mitiglinide) in Phase III clinical trial in this class (Chakrabarti and Rajagopalan 2002).

Repaglinide is a suitable option for patients with severe sulpha allergy who are not candidates for sulfonylurea therapy. The drug is used as monotherapy or in combination with metformin. The major side effects are weight gain, gastrointestinal disturbances and hypoglycaemia (Scheen 1997).

1.2.7.7 Advancements in Diabetes Management

1.2.7.7.1 Prevention of Autoimmune attack

There are several attempts made to control autoimmune attack on the $\beta$-cells and there are several ongoing diabetes prevention trials worldwide. Generally it is preferable to start a specific immuno-modulatory treatment while substantial $\beta$-cells mass remains; that’s during the prediabetic phase (Herold and Rubenstein 1988; Knip and Akerblom 1999). The vitamin B-complex nicotinamide is currently undergoing a multicentre trial in Europe. Nicotinamide
is thought to protect against damage acting as an antioxidant and thus inhibits the deleterious effects of free radicals. It also inhibits the enzyme Poly (ADP-ribose) polymerase (PARP), thereby saving the cellular stores of Nicotinamide adenine diphosphate (NAD). Furthermore, it stimulates islet cell proliferation (Sandler and Anderson 1988). Another interesting immunosuppressive compound, which has shown encouraging results in newly diagnosed patients, is cyclosporine A, which acts by inhibiting T-helper lymphocyte function (Skyler 1987). Unfortunately cyclosporin A must be given early and it has potentially serious side effects, including a toxic action on the β-cell itself (Sandler and Anderson 1988).

Newer immunosuppressive drugs, such as FK-506 Transpl, are under investigation, and some of these side effects may be avoided. Moreover, Bacillus Calmette-Guerin (BCG), a non-specific immunostimulant, has been shown to induce extended remission in newly diagnosed patients by unknown mechanism (Wright et al. 1999).

1.2.7.2 Transplantation

Transplanting technology of either the pancreas or preparations of islet tissues is limited by the problem of obtaining donor tissue and preventing immune rejection of the graft (Sutherland 1989). Nevertheless, transplanting is as yet the only available treatment that can lead to insulin independence. Human allograft transplantation can not be used on a large scale in clinical practice. After whole pancreas transplantation the graft survival after one year is 85-90%. Islet transplants are much more vulnerable. Many of them fail within few weeks or months after engraftment and most islet transplants (> 90%) fail within one year (Sutherland 1989). The reasons for these functional failures are largely unknown, although insufficient numbers of islets, engraftment difficulties, chronic rejection and recurrence of autoimmune disease have been suggested to be contributing factors. Moreover,
hyperglycaemia in the recipient after transplantation deteriorates islet graft survival and function (Landgraf 1996).

One of the major obstacles for clinical islet transplantation is lack of donors. Therefore, it is important to optimize the number of β-cells harvested from each donor, stimulate the growth and/or differentiation of β-cells or to genetically manipulate insulin producing cell lines for transplantation (Sutherland 1989). The differentiated β-cells have the ability to proliferate at a low pace. The proliferation rate can be affected in many ways, for example, by growth stimulating hormones like growth hormone and prolactin. Also the size and composition of the graft and the blood glucose level in the recipient are of crucial importance for β-cell replication (Sutherland 1989).

1.2.7.8 Herbal Management

The drawbacks of conventional therapy though effective, have led to seeking alternative therapy in herbal medicine. More than 400 traditional plant treatments for diabetes mellitus have been recorded, but only a small number of these have received scientific medicinal evaluation to access their efficacy. Traditional treatments have mostly disappeared in societies, but some are prescribed by practitioners of alternative medicine or taken by patients as supplements to conventional therapy. However, plant remedies are the mainstay of treatment in under developed regions.

A hypoglycaemic action from these treatments has been confirmed in animal models and non-insulin dependent diabetic patients, and various hypoglycaemic compounds have been identified (Bailey and Day 1989). Traditional plant remedies are used throughout the world for a range of diabetic presentations (Ivorra 1989; Bailey et al. 1995). Some plant remedies have class of compounds, some of which have anti-hyperglycaemic potentials, which include flavanoids and alkaloids.
1.2.7.8.1 Anti-hyperglycaemic Class of Compounds

1.2.7.8.1.1 Flavanoids

Flavanoids such as quercetin are inhibitors of insulin receptor tyrosine kinase-catalyzed phosphorylation of a glutamic acid-tyrosine random copolymer, while insulin stimulated autophosphorylation of the receptor itself. In adipocytes, quercetin inhibits malate dehydrogenase, lactic dehydrogenase and pyruvate kinase; hence, quercetin inhibits glucose transport, oxidation, and incorporation into lipids (Shisheva and Shechter 1992). Myricetin has insulinomimetic properties and this polyhydroxylated flavonol stimulates lipogenesis and glucose transport in the adipocytes. This compound has no effect on insulin receptor autophosphorylation or glucose uptake. Another flavanol, genistein, increases glucose-stimulated insulin release. Hence, these compounds might play a role in the management of non-insulin-dependent diabetes mellitus (Elliott et al. 2000).

1.2.7.8.1.2 Alkaloids

1-Ephedrine is the major alkaloid component that is known to suppress hyperglycemia. Alkaloid 1-ephedrine promotes the regeneration of pancreas islets following atrophy, restoring the secretion of insulin, and thus correct hyperglycemia. This can be used in the management of diabetes mellitus (Elliott et al. 2000). However, these plants are highly toxic due to the presence of toxic compounds and trace metals.

1.2.7.8.2 Toxicity of Some Class of Compounds

1.2.7.8.2.1 Flavanoids

The flavanoids possess anti-inflammatory, antioxidant, anti-allergic, hepatoprotective, anti-thrombotic, antiviral and anticancer properties. They also have "anti-aging" properties possibly through their antioxidant activity (Elliott et al. 2000). Flavanoids are typical
phenolic compounds and therefore, act as potent metal chelators and free radical scavengers. They are powerful chain-breaking antioxidants. The flavanoids display a remarkable array of biochemical and pharmacological actions, hence potentially health promoting, and disease-preventing compounds (Torel et al. 1986).

Some flavonoids like hydroxyl flavans are capable of quinone formation, a familiar pathway leading to contact sensitization. However, the flavanoids are not potent contact allergens and are not distinguished as contact sensitizers in the dermatologic literature, even though essentially all human beings have daily physical contact with flavonoid-containing foods and plants. Some flavonoids and their related phenolic compounds have toxic effects. However, such flavonoids are not found in human food supply and adverse reactions to flavonoids in humans are rare (Elliott et al. 2000).

1.2.7.8.2.2 Alkaloids

Alkaloids derived from 1-hydroxymethyl-1, 2-dehydropyrrolizidine and esterified with at least one branched C₅ carboxylic acid like pyrrolizidine alkaloids (PAS) display a toxic, carcinogenic and mutagenic effect. Enhancement of the effect occurs if a further hydroxyl group is introduced into the 7-position and also esterified (Zeinsteger et al. 2003).

Pyrrolizidine alkaloids (PAS) are metabolized in the liver by means of hydrolysis, N-oxidation and demethylation. Metabolites derived from N-oxidation process mainly affect the liver leading to intense cellular alterations known as megalocytosis (Zeinsteger et al. 2003). Alkaloid N-oxides, on principle, display the same toxicity. Alkaloids lead to megalocytosis, fatty degeneration, proliferation of the biliary tract epithelium, liver cirrhosis, nodular hyperplasia and adenomas or carcinomas. The clinical symptoms usually occur suddenly and include, colicky pains in epigastrium, vomiting and bleeding diarrhea, ascites formation
within several days, and enlargement of the liver, and hematemesis. In addition, they also affect lungs and kidneys, small intestine and central nervous system (Zeinsteger et al. 2003).

1.2.7.8.2.3 Terpenoids

Volatile substances like terpenes and indoles are part of the essential oils found in plants. Terpenes are also low-toxicity components. These kinds of components can alter membrane permeability. Complex esters of sesquiterpenes alcohols and aromatic acids cause an increment in membrane permeability to divalent and monovalent ions. The final consequence is the disruption of the ionic equilibrium between intra- and extra-cellular compartments (Zeinsteger et al. 2003). For example, β-myrcene, a monoterpenic, causes skeletal alterations, hepatic and renal injuries (De-Olivera et al. 1997).

Symptoms are principally gastrointestinal due to gastric irritation after oral ingestion. Sometimes chemical pneumonitis is possible because terpenes are absorbed in intestine and after distribution they remain in lung tissue (Zeinsteger et al. 2003). Sesquiterpenes alter permeability to cations at the lipidic bilayer of cell and mitochondrial membranes. β-myrcene is toxic for the stomach and liver after its oral administration. It is also highly irritant to the peritoneum and deaths after intraperitoneal injection are possibly due to drug induced chemical peritonitis. Terpenes also lead to necrosis of small and large intestines and in the liver (Zeinsteger et al. 2003).

1.2.7.8.2.4 Saponins

Saponins are strong laxatives because they are poorly absorbed in the gastrointestinal tract. An overdose induces bloody diarrhoea. In the small intestines, saponins lead to acute erosion of the superficial or middle parts of the intestinal villi and lead to haemorrhage inside the lamina propria. In the liver, they cause haemorrhage in many lobules and congestion of
central veins and liver sinusoids hence necrosis of liver cells. In the kidneys, saponins lead to haemorrhage in the glomeruli and focal destruction of the renal tubules. Toxic levels will therefore cause cardiac failure, acute hypoglycaemia and hepatorenal damage leading to death (Diwan 2000).

1.2.7.8.2.5 Tannins

Naturally occurring tannins like hydrolysable tannin, gallotannin, possess anti-tumour promotion activity (Gali-Muhtasib et al. 2001). However, tannins in plants inhibit insect growth and disrupt digestive events in ruminants (Marjorie 1999).

1.2.7.8.2.6 Anthraquinones

The naturally occurring anthraquinones like alizarin (1,2-dihydroxyanthraquinone) and lucidin (1,3-dihydroxy-2-hydroxymethylanthraquinone) are only known to have anti-tumour activity (Itokawa et al. 1993). Hence, they are potential in the development of anti-cancer agent. However, some anthraquinones like lucidin (1,3-dihydroxy-2-hydroxymethyl-9,10-anthraquinone) and its derivatives are cytotoxic and genotoxic (Westendorf et al. 1988).

1.2.7.8.2.7 Sterols

Plant sterols are structurally similar to cholesterol and are often called phytosterols. β-sitosterol, campesterol and stigmasterol are the most abundant. Plant sterols are poorly absorbed; however, clinical studies show that plant sterols and stanols may lower total plasma cholesterol concentrations (Marjorie 1999).

Plant sterols decrease total serum cholesterol and LDL cholesterol concentrations in a dose dependent manner, but not serum high-density lipoproteins (HDL) cholesterol. The
mechanism by which plant sterols lower cholesterol has not been fully determined. However, it is proposed that they compete efficiently with dietary cholesterol for micelle incorporation, reduce cholesterol transport across the unstirred water layer in gastrointestinal tract and lastly, inhibit cholesterol esterification in enterocytes. Since plant sterols and stanols may alter micelle formation, it is possible that absorption of fat-soluble nutrients (such as vitamin E and carotenoids) is diminished. β-sitosterol suppresses tumour cell growth (Marjorie 1999).

1.2.7.8.3 Toxicity of Trace Elements

Human body contains copper bound to tissue proteins. Some of these proteins include, cerebrocuprein I, erythrocuprein, heamocuprein, hepatocuprein and mitochondrocuprein. Some copper proteins have enzymatic activities like ceruloplasmin, tyrosinase, amino oxidase, cytochrome C oxidase, urease and dopamine-β-hydroxylase. Flavoproteins generally require copper for proper functioning. Copper act as a catalyst in some stages of haemoglobin synthesis (Ouma 1994). Excessive amounts of copper levels are characteristic of a number of diseases. Free copper interferes with certain enzymatic systems, notably membrane adenosine triphosphatase and the lipoic acid-pyruvate oxidase system. A well known pathological condition in man manifested by excessive body stores of copper is hepatolenticular degeneration, or Wilson's disease. This disease is associated with the failure of the liver to remove albumin-bound copper from the plasma and incorporate the copper into ceruplasmin. This defect is thought to be due to the genetically determined absence of a liver enzyme system. The symptoms of toxicity are diarrhea, epigastric pain and discomfort, blood in the urine, liver damage, low blood pressure and vomiting (Ouma 1994).

Lead is required for normal growth in animals. However, kidney is the main organ damaged by long-term lead toxicity. It leads to nephrotoxicity hence renal tubular
dysfunction (Singh et al. 1999). Toxicity from zinc supplements can cause renal failure following haemodialysis characterized by flu-like symptoms, fever, epigastric pain, fatigue, vomiting, dehydration, anemia, depressed immune function and decrease in the "good" form of cholesterol. Excessive zinc interferes with the function of copper and iron (Ouma 1994). Toxicity of manganese include dementia, psychiatric disorders similar to schizophrenia, and neurological changes resembling Parkinson's disease (Friedman 1987). On the other hand excessive toxicity of molybdenum for prolonged periods causes headache, irritability, lethargy and coma. It also interferes with copper and iron metabolism (Reul et al. 1997).

Iron poisoning is the most common cause of death resulting from poisoning in children. Supplemental iron can cause gastric irritation, abdominal pain, constipation, diarrhoea, nausea and vomiting. Certain antacids may decrease the absorption of iron supplement (Jose' Manuel et al. 2002). Although magnesium is important in diabetes management, high levels impairs kidney function (McNair et al. 1978).

1.2.7.9 Plants Used in the Management of Diabetes Mellitus

1.2.7.9.1 Onion (Allium cepa) and garlic (Allium sativum)

Onion (Allium cepa) and garlic (Allium sativum) contains active hypoglycemic constituents. Garlic (Allium sativum) also contains a hypoglycemic organic sulphur compounds (Rawi et al. 1998). Volatile oils in raw onion and garlic cloves lower fasting glucose concentration in both diabetic animals and human subjects (Jain et al. 1973). The active components are believed to be sulfur-containing compounds such as allyl propyl disulfide (APDS) in onions and diallyl disulfide (allicin) in garlic. These active ingredients lower glucose levels by competing with insulin (which is also a disulfide) for insulin-inactivating sites in the liver, resulting in an increase of free insulin. Onion extracts reduce blood sugar levels in a dose dependent manner. A typical dosage of Allium cepa is one
400mg capsule daily while the general dosage of garlic is 4g fresh garlic or 8mg of the essential oil (Sharma et al. 1977).

1.2.7.9.2 Green Tea (Camellia sinensis)

Green tea (leaves of *Camellia sinensis*, Theaceae) is a popular beverage in Kenya and East Asia, and also used as a herbal remedy in Europe and North America. Green tea is considered to be anti-inflammatory, anti-oxidative, anti-mutagenic, and anti-carcinogenic and can prevent cardiac disorders. Epidemiologically, it has been suggested that green tea consumption prevents type 2 diabetes (Hiroshi et al. 2004). Green tea extract contains polyphenols like, catechin, epicatechin, epigallocatechin, and their gallates, tannin and caffeine. Furthermore, the polyphenols in green tea extract have epigallocatechin-3-gallate as the main constituent with anti-diabetic activity (Broadhurst et al. 2000). The extract also has pyrroloquinoline quinone, a newly discovered vitamin (Kasahara and Kato 1993). Some constituent components enhance the basal and insulin-stimulated glucose uptake, inhibit intestinal glucose uptake by inhibiting the sodium-dependent glucose transporter in the intestinal epithelial cells, and reduce serum glucose level in alloxan-diabetic rats (Sabu et al. 2002). Controversially, caffeine acutely lowers insulin sensitivity in humans (Keijzers et al. 2002).

1.2.7.9.3 Panax Ginseng (Panax quinquefolius)

Panax ginseng (*Panax quinquefolius*) is widely used in Chinese medicine for over 2000 years. It has been used for the treatment of a variety of conditions and hence considered atonic to elevate mood and reduce fatigue. Other pharmacological properties including immunostimulation and liver protection were also reported (Gehan 2001).
The root of ginseng has been used for over 2,000 years in the Far East for its health promoting properties. Of the several species of ginseng, *Panax ginseng* (Asian ginseng) and *Panax quinquefolius* (American ginseng) are commonly used. Constituents of all ginseng species include ginsenosides, polysaccharides, peptides, polyacetylenic alcohol, and fatty acids. Most pharmacological actions of ginseng are attributed to ginsenosides, a family of steroids named steroidal saponins (Attele *et al.* 1999). The chemical composition of ginseng products and potency may vary with the plant extract derivative, the age of the root, the location where grown, the season when harvested, and the methods of drying. Both Asian and American ginseng has significant hypoglycemic action. The blood lowering effect appears to be attributed to ginsenoside Rb-2 and more specifically to panaxans I, J, K and L. But whether these constituents have a similar effect on type 2 diabetes is yet unknown (Liu and Xiao 1992).

The ginseng's mechanisms of action are thought to be: slowing the digestion of food, decreasing the rate of carbohydrate absorption into portal hepatic circulation; ginseng may affect glucose transport, which is mediated by nitric oxide (NO); and lastly, ginseng may modulate NO-mediated insulin secretion and NO stimulates glucose-dependent secretion of insulin (Gillis 1997).

However, the side effects of ginseng are nervousness and excitation. It also exerts an estrogen-like effect in postmenopausal women, resulting in diffuse mammary nodularity and vaginal bleeding. It may also inhibit the effects of warfarin and interact with the monoamine oxidase inhibitor phenelzine. Massive overdose causes ginseng abuse syndrome, which is characterized by hypertension, insomnia, hypertonia, and edema (Jones and Runikis 1987). The recommended daily ginseng dosage is 1-3 g of the crude root, or 200-600 mg of a standardized extract (Jones and Runikis 1987).
1.2.7.9.4 Bitter Gourd (*Momordica charantia*)

Bitter Gourd (*Momordica charantia*), also known as balsam pear is a tropical vegetable widely cultivated in parts of Asia, Africa, and South America, which has been extensively used in folk medicine as a remedy for diabetes. This is a climbing herb and also common in Kenya. The active, hypoglycemic constituents include charantin, obtained from an alcohol extract of the fruit, and a polypeptide called p-insulin (plant insulin or polypeptide-p) isolated from the fruit and seeds of the plant. The p-insulin consists of 166 residues containing 17 amino acids and has a molecular weight of 11,000. It is structurally and pharmacologically comparable to bovine insulin, and is composed of two polypeptide chains with disulfide bonds. p-insulin has an onset of action similar to bovine insulin (30-60 minutes) and a peak hypoglycemic effect after 4 hours in type I diabetics, compared with 2-3 hours for regular insulin. Although the precise mechanism of action remains to be fully elucidated, *M. charantia* stimulates insulin release or possibly glycogen synthesis in the liver (Welihinda *et al.* 1986). Additionally, the plant is believed to contain several anti-diabetic principles. The hypoglycemic effects of this plant appears to be due to extra-pancreatic activity, including increased glucose utilization by the liver (Sarkar *et al.* 1996); decreased glucose synthesis by depression of key gluconeogenic enzymes like glucose-6-phosphatase and fructose-1, 6-biphosphatase; and enhancement of glucose oxidation through the shunt pathway via activation of glucose-6-phosphate dehydrogenase (Shibib *et al.* 1993).

Interestingly, these herbs on an individual basis are reported to possess a variety of healthful properties, including blood glucose regulating, immunomodulating, liver detoxifying, and anti-inflammatory properties. These properties are significant to the diabetic as autoimmune processes are believed to play a role in the destruction of β-cells, and inflammation mediated by free radicals is also characteristic of the diabetic condition (Tim 1998).
The recommended dose of bitter melon depends on the form it is being consumed. Dosage for tincture ranges from 5 mL two to three times daily to as high as 50 mL per day. However, bitter melon juice is very difficult to make palatable since, as the name implies, it is quite bitter. To avoid the bitter taste, the Indians and Chinese crush the herbs and form tablets. In Central America, it is prepared as an extract or decoction (Dey et al. 2002).

Dosage of capsulized dried powder ranges from 3-15g daily. That is quite a large dose so to avoid the necessity of taking so many capsules; a standardized extract may be used at dosages of 100-200 mg three times daily (Dey et al. 2002).

1.2.7.9.5 Ackee fruit (*Blighia sapida*)

Ackee is the National fruit of Jamaica and was imported from West Africa in the 18th century. It is a tall, leafy tree (up to 12 meters) that produces clusters of fruits widely used for human consumption and for industrial purposes. The fruit is yellow in color and shaped like an oblong capsule that contains three cream-colored arils. The arils may be consumed safely when the fruit becomes red and opens under the light of the sun. It is then commonly boiled in water or milk and eaten alone or in meat or fish dishes. It is also consumed raw in some African countries. When ingested unripe, ackee produces vomiting and fatal cases of poisoning (Odutuga et al. 1992).

Ackee fruit contains hypoglycin, a natural toxin. It exists as a cyclic amino acid, hypoglycin A (HG-A), and its gamma-glutamyl derivative, hypoglycin B (HG-B). When the fruit is consumed unripe, it produces an acute toxic effect within two to three hours with symptoms including nausea, vomiting, headache and drowsiness. Coma and death may occur within 12 hours in severe cases. The most toxic is hypoglycin A, which is found in the unripe arils. Hypoglycin A is a water-soluble liver toxin that produces hypoglycemia through the
inhibition of gluconeogenesis, secondary to the limitation of cofactors (CoA and carnitine) that are essential for the oxidation of long-chain fatty acids (Odutuga et al. 1992).

The concentration of hypoglycin A in the unripe ackee is 20 times greater than in the mature fruit, however the level of concentration of the toxin lowers rapidly after its exposure to the sun. The seeds contain hypoglycin B and are always poisonous. An important factor seems to be the nutritional status of the person consuming ackee, since diagnosed patients often present chronic malnutrition and vitamin deficiencies (Odutuga et al. 1992).

1.2.7.9.6 Fenugreek (Trigonella foenum graecum)

*Trigonella foenum graecum* has been used as a remedy for diabetes, particularly in India. The active principal is in the defatted portion of the seed, which contains the alkaloid trigonelline, nicotinic acid and coumarin. Administration of the defatted seed (1.5-2.0 g/kg daily) reduces fasting and postprandial blood levels of glucose, glycagon, somatostatin, insulin, total cholesterol, and triglycerides and increased HDL-cholesterol levels (Dey et al. 2002).

Human studies have confirmed the glucose and lipid lowering effects. The fiber constitutes potential mechanisms of fenugreek’s beneficial effect in diabetic patients. Dosages of the fiber range from 10-100g daily in divided dosages. The major side effect is that the urine may have a maple syrup smell after fenugreek consumption (Dey et al. 2002).

1.2.7.9.7 Gurmar (Gymnema sylvestre)

*Gymnema sylvestre*, a plant native to the tropical forests of India, has long been used as a treatment for diabetes. It is postulate that *Gymnema sylvestre* enhances the production of endogenous insulin. A typical dosage of *Gymnema sylvestre* extract is 400-600 mg/day. One of its side effects may be a reduction or loss of the taste sensation of sweetness
and bitterness, although this occurs only if the plant is directly exposed to the tongue (Dey et al. 2002).

1.2.7.9.8 Bilberry (*Vaccinium myrtillus*)

*Vaccinium myrtillus* is a shrubby plant that grows in Europe. Leaves of bilberry were widely used as a treatment for diabetes before availability of insulin. Oral administration of bilberry leaf tea reduces blood sugar levels in diabetics. It has also a beneficial effect in microvascular abnormalities of diabetes particularly retinopathy. In the case of vascular complications, however, the fruit rather than the leaf are used, with the anthocyanosides being the most important constituent (Dey et al. 2002).

The standard dose of the fruit extract is based on its anthocyanosides contents and is 80-160 mg three times daily of a 25% anthocyanoside extract. The ideal dosage of bilberry leaf for lowering blood sugar has not been elucidated (Dey et al. 2002).

1.2.7.9.9 Salt Bush (*Atriplex halimus*)

*Atriplex halimus* (salt bush) is a plant native to Israel, where much of the clinical data has been collected. Small animals called sand rats develop type 2 diabetes when deprived of this plant. The data on its use for type 2 diabetes in humans is limited to unpublished reports in which 3g/day decreased blood glucose levels (Dey et al. 2002).

1.2.7.9.10 Aloe vera

The dried sap (fluid) of Aloe vera is a traditional remedy used for diabetes in the Arabian peninsula. *Aloe vera* juice is prepared from *Aloe vera* gel, a mucilaginous preparation obtained from the leaves of the plant. Oral administration of the juice reduces fasting blood glucose and triglyceride levels in type 2 diabetic patients with or without
combination of a conventional anti-diabetic agent. The amount used is one tablespoon of *Aloe vera* juice with no significant adverse effects reported (Dey *et al.* 2002).

1.3 Plants of this Study

Seven plant species were studied for hypoglycaemic activity and include; *Terminalia brownii*, *Olea africana*, *Pentas zanzibarica*, *Warburgia salutaris*, *Aloe secundiflora*, *Rumex abyssinicus* and *Rhoicissus tridentata*. These plants were selected based on ethnobotanical information from traditional medicine practitioners.

1.3.1 *Rumex abyssinicus* (Dock) Polygonaceae

*Rumex abyssinicus* is a herb that grows to 2 m tall with reddish green hollow stem and terminal panicle of crowded green flowers. Leaves are sheathed, arrow-shaped at the base. Stems are chewed to relieve thirst. This is usually a common plant found in abandoned cultivation usually in wetter places (Gachathi 1989).

It is usually referred to as Mugagatio (Kikuyu), Kinyonywe (Kamba), Omuka (Nyankore), Gentamana (Shambaa) in Enkaiswishoi in Maasai. The soft stems and leaves are pounded together and the expressed sap is used as a treatment for pneumonia. The leaves are used as a cough cure (Kokwaro 1993).

Its roots, which have a yellow dye, are used in treatment of scabies. The dried roots are pounded and applied to wounds, or mixed with water and the infusion drunk as a remedy for stomach-ache (Munavu *et al.* 1984). *R. abyssinicus* has been shown to have antimicrobial, antiviral and anti-fertility properties (Vlietinck *et al.* 1995; Desta 1994).
combination of a conventional anti-diabetic agent. The amount used is one tablespoon of Aloe vera juice with no significant adverse effects reported (Dey et al. 2002).

1.3 Plants of this Study

Seven plant species were studied for hypoglycaemic activity and include; Terminalia brownii, Olea africana, Pentas zanzibarica, Warburgia salutaris, Aloe secundiflora, Rumex abyssinicus and Rhoicissus tridentata. These plants were selected based on ethnobotanical information from traditional medicine practitioners.

1.3.1 Rumex abyssinicus (Dock) Polygonaceae

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1.3.2 *Olea africana* (Mill.) P.Green. Oleaceae

*Olea africana* is a shrub or tree, which grows 3-24 m tall, bole often gnarled; the bark is grey or dark brown and longitudinally fissured. The leaves are glossy dark green above, dull or metallic golden or silvery beneath. The flowers are many, in terminal and lateral paniculate cymes and are usually white or creamy yellow and scented. The fruits are purple or black in colour. It is found in dry upland evergreen forest and also in woodland on lava flows. It is known as Mutamaiyu, Mutero (Kikuyu), Ol-Orien (Maasai), Muthata (Meru), Molialundi (Kamba) and in Lorien (Dorobo) (Beentje 1994).

The bark of the tree is pounded, soaked in water and left overnight. The infusion is then drunk for treating tapeworm. The bark decoction is drunk as an anthelmintic, or decoction used in steam bath and some drunk for treatment of itchy rash. The leaf is also drunk for hepatic diseases (Kokwaro 1993). Studies also show that *O. africana* has antihypertensive activity (Simon and Lamla 1991).

1.3.3 *Warburgia salutaris* (Bertol.f) Chiov. Canellaceae

This is a tree that grows 40 m tall but often-quite short (about 10 m) with blackish rough bole and rounded compact crown of shiny dark green leaves. Flowers are greenish while fruits are greenish purple and egg shaped (Gachathi 1989). This tree is found in riverine forest and drier upland forest. It is also now rare around Nairobi, due to its overexploitation (Beentje 1994).

*W. salutaris*’ local name is Kenya greenheart. It is also known as Muthiga (Kikuyu), Apacha (Luhya), Olosogoni (Maasai), Soget (Kipsigis) and Sogo-Maitha (Luo). The bark, which is hot to the taste, is used in the treatment of chest pains, fevers and toothaches. This is a common tree in dry forest (Gachathi 1989). The dried bark is also chewed and acts as remedy for stomach-ache, constipation, coughs, muscle pains, weak joints, and general body
pains. A decoction of the bark or leaves is taken as a cure for malaria, but causes violent vomiting (Kokwaro 1993).

1.3.4 *Terminalia brownii* Fres. Combretaceae

*Terminalia brownii* is a shrub or tree, which grows to 2.5-20 m tall. It has greyish-brown fissured bark. Leaves are spirally arranged, flowers are white or cream, in spikes of 12 m long and fruits are reddish purple. This shrub is found in deciduous bush land or woodland, often on rocky outcrops, or riverine. It is usually known as Kuuku, Muvuku (Kamba), Onera (Luo), Mururuku (Embu) and Ekuyen (Turkana).

The bark decoction is used as remedy for fevers and colds by the Turkana and the Pokot (Beentje, 1994). The phloem fibres are chewed and the solution swallowed in the treatment of yellow fever particularly in children (Kokwaro 1993). *T. brownii* is known to have antibacterial activity (El Fatih et al. 1998).

1.3.5 *Pentas zanzibarica* (Kl.) Vatke. Rubiaceae

The local names are Mdimi (Shamba) and Mdobe (Digo). The juice of the pounded leaves, mixed with little water, is drunk as a drastic purgative. A decoction of the roots is taken as a remedy for gonorrhoea and syphilis. Roots and leaves are also boiled to make a form of tea, which is given to children as a tonic (the decoction of the roots may be mixed with skimmed milk) (Kokwaro 1993). In Tanzania, the root is used with other plants in the treatment of cerebral malaria, headaches, rheumatic pain, gonorrhea and syphilis (Chhabra and Mahunnah 1994).
1.3.6 *Aloe secundiflora* Engl. Liliaceae

It is also known as Kiluma (Kamba), Sukoroi (Samburu). It is used in treatment of malaria, headache, pneumonia, conjunctivitis (where a drop of the sap is applied into the eyes), chest pains, and for disinfectant purposes. It is also used with *Pappea capensis* and *Fagara chalybea* and the decoction obtained used for the treatment of internal body swellings known as “kati” (Kamba) (Kokwaro 1993).

1.3.7 *Rhoicissus tridentata* (L.f.) Wild & Drum. Vitaceae

This is a shrub or a climber, which grows to a height of 1.5 m to 9 m. The leaves are trifoliolate and hairy. The fruits are black in colour and about 1 cm in diameter. It is found in bush land, usually on rocky hillsides and also in drier forest margins. It is known as Mutumutua (Kikuyu), Kivosya Nguguu (Kamba), Durutua (Luhy), Oronymbeche, Egesanga (Kisii), Dorodwet (Kipsigis), Ol-Kilenyei (Maasai) and Iwambova, Torotwa (Marakwet), Taratwo (Pokot), Mgeruguru (Taita) and Mutonganego (Hehe) (Beentje 1994).

The root decoction is anthelmintic, but overdosing may be fatal. The sap from the branches is antiseptic (Beentje 1994). It is applied to cuts and sores as it has anaesthetising (numbing) properties. The roots are chewed and the juice swallowed to ease indigestion, and to cure abdominal pains during menstruation (Kokwaro 1993).

In Tanzania, fresh stems are used to promote healing of cuts and sores and also to anaesthetize cuts and sores (Chhabra et al. 1984). In South Africa, its dried leaf and stem are used as an anti-inflammatory agent (Lin et al. 1999). In Kenya, it has been found to have anti-malarial activity (Gakunju et al. 1995).
1.4 Justification for this study

Diabetes mellitus has attained epidemic proportions worldwide today and is a very expensive disorder to manage by use of conventional drugs. Conventional drugs are either unavailable or unaffordable and may still have undesirable side effects. African communities still rely mainly on herbs as a source of primary health care needs. Herbal medicines are cheap and readily available from traditional healers but it is important to investigate their efficacy in management of diabetes as well as their toxicity. Due to an increase in human activities, plant species are disappearing everyday hence it is important to study their importance while they are still available and conserve the flora.

1.5 Hypothesis

(i) Medicinal plants used in herbal medicine by the traditional healers to treat diabetes have hypoglycaemic activities.

(ii) Medicinal plants used by traditional healers are toxic to human beings.

1.6 Objectives

1.6.1 Main Objective

- To screen the seven selected medicinal plants for their antihyperglycaemic activity and their safety.

1.6.2 Specific Objectives

(i) To screen the extracts for in vivo hypoglycaemic activity.

(ii) To determine the trace elements in the extracts.

(iii) To determine the class of compounds in the extracts.

(iv) To evaluate the toxicological effects of the extracts
Chapter Two
Materials and Methods

2.1 Medicinal plants

The following 7 medicinal plants were investigated for anti-diabetic activity. The medicinal plants were chosen based on ethno botanical information (Simon Mathenge Itegi, Personal communication).

Table 1: Botanical, family names and parts of the plants used

<table>
<thead>
<tr>
<th>Name of the plant</th>
<th>Family</th>
<th>Part of the plant used</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aloe secundiflora.</em> Engl.</td>
<td>Liliaceae</td>
<td>Leaves</td>
</tr>
<tr>
<td><em>Olea africana.</em> Mill. P, Green.</td>
<td>Oleaceae</td>
<td>Leaves</td>
</tr>
<tr>
<td><em>Pentas zanzibarica.</em> (Kl.)Vatke</td>
<td>Rubiaceae</td>
<td>Leaves</td>
</tr>
<tr>
<td><em>Rhoicissus tridentata.</em> (L.f) Wild and Drum</td>
<td>Vitaceae</td>
<td>Root tubers</td>
</tr>
<tr>
<td><em>Rumex abyssinicus.</em> Jacq</td>
<td>Polygonaceae</td>
<td>Leaves and stems</td>
</tr>
<tr>
<td><em>Terminalia brownii.</em> Fres.</td>
<td>Combretaceae</td>
<td>Roots</td>
</tr>
<tr>
<td><em>Warburgia salutaris.</em> (Bertol.f.) Chiov.</td>
<td>Canellaceae</td>
<td>Stem bark</td>
</tr>
</tbody>
</table>
2.1.1 Collection of the plants

The plant species used in the present study were collected from their natural habitats in different geographical zones of Kenya. *Terminalia brownii, Olea africana, Pentas zanzibarica* and *Aloe secundiflora* were collected from Gachoka Division, Mbeere District, in Eastern Province. *Rhoicissus tridentata* was collected from Laikipia District, Rift Valley Province. However, *Rumex abyssinicus* was collected from Chiromo campus, University of Nairobi while *Warburgia salutaris* was collected from Kenyatta University. A plant taxonomist (Mr Geoffrey Mungai) identified the plant samples and voucher specimens were deposited in the East African Herbarium, Nairobi, Kenya.

2.1.2 Initial processing of the plants

The roots, leaves and stems were harvested and their barks peeled off while still fresh and cut into small portions and dried at room temperature for one month. The leaves were collected while green and dried similarly. The plant sample specifically the roots, stems, bark, and leaves were ground into powder form using an electric mill (Christy and Norris Ltd., England). The powdered plant materials were kept separately in closed plastic containers at room temperature.

However, *Rumex abyssinicus* was used while fresh. The leaves and stems were crushed in a stainless steel mortar and squeezed by means of a fine cloth to separate the juice. The juice was then filtered through Whatman No 42 micro fibre filter paper kept in a clean plastic container in a refrigerator ready for freeze-drying.

2.1.3 Preparation of the aqueous extracts

One hundred grams of each powdered plant material was boiled in 1 litre of distilled water for 1 hour. At the end of boiling time, the extract was decanted into a clean dry conical
flask and then filtered through folded cotton gauze into another dry clean conical flask. The filtrate was then freeze-dried in 200 ml portions using a Modulyo Freeze Dryer (Edwards, England) for 48 hours. The freeze-dried powder was then weighed and stored in an airtight container at -20°C until used for bioassay.

2.1.4 Animals

Male Swiss albino mice (3-4 weeks old) bred in the animal house of Biochemistry and Biotechnology Department of Kenyatta University were used. These mice were weighing 22 - 25 g. The mice were housed at a temperature of 25°C with 12 hours /12 hours darkness photoperiod and fed on rodent pellets and water ad libidum.

2.1.5 Experimental induction of hyperglycaemia

Hyperglycaemia was induced experimentally by the administration of alloxan (2,4,5,6 tetraoxypyrimidine; 5-6-dioxyuracil) obtained from Sigma (Steinhein, Switzerland). The mechanism of action is known to be through specific necrosis of pancreatic islets (Szkudelski. 2001). The animals were injected intra-peritoneally with 150 mg/kg body weight of 10% alloxan monohydrate. Three to four days after administration of alloxan, blood glucose level was measured. Mice, which had blood glucose levels above 1000 mg/L, were considered diabetic. They were fasted for 8-12 hours prior to use in bioassay (Szkudelski 2001).

2.1.6 Experimental Design

The following groups of mice were used in the experiment; the normal unmanipilated mice (the reference group of the experiment), alloxan-diabetic control mice (the negative control group of the experiment), alloxan-diabetic control mice, injected with insulin (positive control group) and alloxan-diabetic experimental mice treated with plant extracts.
2.1.7 Preparation of extracts for injection in mice

The appropriate doses of extracts were made by dissolving 62.5 mg (25 mg/kg body weight), 87.5 mg (35 mg/kg body weight), 112.5 mg (45 mg/kg body weight), 125 mg (50 mg/kg body weight), 250 mg (100 mg/kg body weight) and 375 mg (150 mg/kg body weight) in 10 ml physiological saline respectively. Insulin dose was prepared by dissolving 25 insulin units in 100 ml (1 IU/kg body weight) of physiological saline. For injections, 0.1 ml from the solutions prepared were administered intraperitoneally. Blood sampling was done by sterilizing the tail with 10% alcohol and then nipping the tail at start of the experiment and repeated after 1 hr, 2 hr, 3 hr and 4 hr. The blood glucose levels were determined with a glucose analyzer model (Hypoguard, Woodbridge, England).

2.2 Elemental Analysis

2.2.1 Energy Dispersive X-Ray Fluorescence Spectroscopy (EDXRF System)

EDXRF system was used to determine the content of manganese, iron, nickel, copper, zinc, strontium, molybdenum, and lead in the plant extract samples.

2.2.1.1 Preparation of Plant Extracts

Each freeze-dried sample were filtered and weighed. At least two pellets weighing 300-1000 mg/cm$^2$ were prepared for analysis using the press pellet machine (Holynska 1993).

2.2.1.2 EDXRF system

The energy dispersive X-ray fluorescence analysis system consists of an x-ray spectrometer and a radioisotope excitation source. The radiation from the radioactive source, Cd$^{109}$ (half life, $T_{1/2} = 453$ 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,
30 mm² × 10 mm sensitive volume, 25 μm Be window) with a energy resolution of 200 eV at 5.9 keV Mn Kα - line. The spectral data for analysis were collected using personal computer based Canberra S-100 multi-channel analyser (MCA). The acquisition time applied in the EDXRF measurement was 1000 seconds. For data analysis, the X-ray spectrum analysis and quantification was done using IAEA QXAS software (QXAS 1992), which is based on the fundamental parameters method (FPM) (Giauque et al. 1973; Sparks 1975).

2.2.2 Atomic Absorption Spectrometry

This method was used to determine the quantity of magnesium, chromium and vanadium in the plant extracts. Atomic absorption spectrophotometer (AAS) Model: 210VGP (Scientific Equipment) was used.

2.2.2.1 Cleaning of Glassware and Plastic Containers

All glassware, which included beakers, funnels, volumetric flasks, measuring cylinders, pipettes and burettes were thoroughly cleaned. This was done by soaking them in a mixture of concentrated nitric acid and concentrated hydrochloric acid (1: 3: 2), followed by overnight soaking in a detergent and subsequent rinsing with distilled deionised water. This was done every time before analysis. Apart from the plastic containers, the glassware were dried in an oven maintained at a temperature of 105 °C for 2 hours, then removed and kept in a dry place.

2.2.2.2 Chemical Reagents

High purity grade reagents were obtained from British Drug House (BDH Analar grade).
2.2.2.2.1 Preparation of Reagents

2.2.2.2.1.1 Standard Stock Solutions

Chromium and Magnesium standard stock solutions of 1000 ppm for atomic absorption spectrophotometer were used as supplied by the manufacturers (Aldrich Chemical Co., Inc). 1.7852g of Vanadium pento-oxide was dissolved in minimum amount of concentrated sulphuric acid and then heated to dissolve completely, then cooled. The solution obtained was made to 1 litre in a volumetric flask with distilled deionised water. This gave 1000 ppm of the stock solution.

Immediately each standard solution was prepared, the flask was thoroughly shaken for proper mixing and the contents then transferred into a clean plastic bottle and kept in a refrigerator.

2.2.2.2.1.2 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 procedure was done for each element when its analysis was due. Hence, during each analysis fresh working standards were prepared. For each element, working solutions were prepared within a given range where the relationship between the concentration and absorbance was linear. In case of magnesium, 2ml 5% lanthanum solution was added to each series of working standards before diluting the standards to volume. In addition, standard blank reagents for Mg, V, and Cr were prepared by adding all the used reagents, except the target element being determined.
2.2.2.1.3 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 volume using distilled deionised water in 250ml volumetric flask. After mixing thoroughly, the solution was kept in clean plastic bottle and used during the determination of magnesium in the plant materials.

2.2.2.3 Digestion of the Plant Materials

Each freeze-dried plant material was brought to solution by wet oxidation. In each case, the procedure was repeated twice.

2.2.2.3.1 Wet Oxidation for Determination of Cr, V and Mg

The dried samples weighing 1g were transferred into 100ml Pyrex beakers and to each beaker, 10ml of concentrated nitric acid was added, then allowed to soak thoroughly. Three millilitres of perchloric acid, (60% HClO₄) was added to each beaker, and then warmed on a 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, 10ml of nitric acid was added and heating continued until white fumes of perchloric acid were observed. The final solution was cooled and 25ml of 20% hydrochloric acid was added. The solution was then quantitatively transferred into 100ml volumetric flask by filtering through Whatman filter paper No. 42. The solutions were then made to volume and shaken well to allow proper mixing before the contents were transferred to plastic sample bottles. For each plant sample, digestion was done in duplicate. The samples were analyzed immediately whenever possible, otherwise kept in refrigerator at -20°C awaiting analysis.
2.2.2.3.2 Determination of Cr, V and Mg Contents by Atomic Absorption Spectroscopy

The wet digests of the plant materials were analysed for Cr, V and Mg. The sample solutions for analysis of magnesium were prepared by withdrawing 1ml of the digested sample solution, into 100ml volumetric flasks. Five millitres of lanthanum solution was added in each flask and the mixture diluted to volume using distilled deionised water. However, for analysis of Cr and V, the digested sample solutions were analyzed without further dilution.

After setting the AAS instrument to the right conditions for each element (Appendix I), the respective standards 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 element, the above procedure was done in duplicate for each sample. 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 and the best fitting straight line was obtained by using simple microprocessor programmes (Appendices II, III and IV). These programmes also helped to convert absorbance readings to concentration of element in each sample analyzed with good accuracy than manual graphical method. The concentration values obtained were corrected by multiplying with the respective dilution factors. The final values were expressed as μg/g dry matter.
2.3 Phytochemical Screening

A phytochemical screening of the plant extracts was done to determine the class of secondary metabolites present which included alkaloids, saponins, tannins, terpenoids and sterols, flavanoids and anthraquinones using standard methods (Houghton and Raman 1998; Hossein and Hani 2002).

2.3.1 Test for Alkaloids

To test for the presence of alkaloids, 2g of each plant extract was stirred in 2ml of 1% aqueous concentrated hydrochloric acid (HCl) and heated in a boiling water bath for 10 minutes. The mixture was then filtered while hot and treated with Dragendorff’s reagent. Turbidity or precipitation was an indication of a preliminary test for alkaloids (Houghton and Raman 1998; Hossein and Hani 2002).

2.3.2 Test for Sterols and Terpenoids

To test for the presence of sterols and terpenoids, 2g of each extract were stirred with n-hexane to remove most colouring materials. The residue was then extracted with 2ml dichloromethane. The dichloromethane solution was dehydrated over anhydrous sodium sulphate. Then 2ml of the dichloromethane portion was mixed with 0.5ml acetic anhydride followed by 2 drops of concentrated sulphuric acid. A gradual appearance of green to blue colour was indicative of sterols. Colour change from pink to purple indicated the presence of terpenoids (Houghton and Raman 1998; Hossein and Hani 2002).

2.3.3 Test for Saponins

To test for the presence of saponins, 1g of the freeze dried plant extract was shaken in 2ml of distilled water in a test tube. The occurrence of frothing which persisted for at least
half an hour was taken as a positive test for saponins (Houghton and Raman 1998; Hossein and Hani 2002).

2.3.4 Test for Flavanoids

To test the presence of flavanoids, 1g of the freeze dried powdered plant sample was defatted by several washing in n-hexane. The defatted residue was washed with 4ml 80% methanol and filtered. The filtrate was used as follows; to 2ml of the filtrate, 1ml of the 1% aluminium chloride in methanol was added. The development of yellow colour was an indication of the presence of flavonols, flavones and/or chalcones. In addition, to 2ml of the filtrate, 1ml of 1% potassium hydroxide was added. The development of a dark yellow colour was an indication of the presence of flavanoids (Houghton and Raman 1998; Hossein and Hani 2002).

2.3.5 Test for Tannins

To assess the presence of tannins, 1g of the extract were stirred in 2ml of distilled water, filtered and 5% ferric chloride added to the filtrate. The appearance of a blue black to green precipitate was evidence for the presence of tannins (Houghton and Raman 1998; Hossein and Hani 2002).

2.3.6 Test for Anthraquinones

To test the presence of free anthraquinones, 2g of the powdered plant materials was shaken with 10ml of benzene and filtered. Five millilitres of a 10% ammonium hydroxide solution was added and the mixture shaken. The presence of a violet colour in the ammoniacal phase was indicative of the presence of free anthraquinones.
To test the presence of bound anthraquinones, 2g of benzene washed plant extract was boiled with 10ml of 1% HCl and filtered while hot. The filtrate was shaken with 10ml benzene. The benzene layer was removed and then 10% ammonia hydroxide added. The presence of a violet colour was indicative of the presence of bound anthraquinones (Houghton and Raman 1998; Hossein and Hani 2002).

2.4 Preliminary Histological Assessment of Toxicity in vivo

For determination of toxicity of plant extracts in normal mice, the mice were divided into two groups. Group I served as a control. It was treated with 0.1ml of physiological saline. Group II was treated with the extract. Each mouse in this group was intraperitoneally injected with the extracts at a dose of 450mg/kgbw. The extracts were administered daily for a period of one month. The animals were kept under close observation and fed on pellet diet and water ad libitum. At the end of thirty days, the mice were sacrificed and pieces of spleen, liver, kidney, heart, lungs, and muscles removed and preserved in 10% formalin.

The tissues were trimmed and washed in running water overnight to remove any excess formalin. The tissues were then processed using an automatic tissue processor; they were dehydrated sequentially in increasing concentrations of alcohols i.e. 50%, 80%, 90%, and 96% at hourly stepped intervals. The tissues were then cleared of alcohol twice in two changes of Xylene. Infiltration with paraffin wax was then done for 3 hours in the paraffin wax oven set at 2°C below the melting point of wax.

The tissues were then embedded in fresh molten paraffin wax and allowed to dry. The embedded tissues were sectioned at 0.5 μm thicknesses with a microtome and floated in warm water to spread out, then attached onto clean microscopic slides. After holding in hot oven for 15 minutes, the tissue sections were dewaxed in Xylene and then stained with haematoxylin and eosin dyes using standard histological protocols. The stained tissues were
cover slipped with DPX, dried and examined microscopically for any pathological changes (Baker et al. 1989).

2.5 Data Analysis

Data was expressed as mean ± standard error of means. Statistical analysis was done by using Student's unpaired t-test to compare the data of untreated group of normal mice with the group of diabetic mice treated with the saline, insulin and the extracts. p<0.05 was considered statistically significant. StatView® and Instat® softwares were used.
Chapter Three

Results

3.1 Effects of aqueous plant extracts on blood glucose levels in alloxan induced diabetic mice

All the three doses (50, 100, and 150 mg/kg body weight) of the aqueous extracts from the stem bark of *Warbugia salutaris* decreased the blood glucose levels in a dose dependent manner. The mice treated with 100 mg extract/kg body weight died after the third hour while those treated with 150 mg extract/kg body weight died after first hour, respectively (Table 2). This decrease in blood glucose was between 60% and 81% in the first hour, 81% and 85% in the second hour, 82% and 85% (with the doses of 50 and 100 mg/kg body weight) in the third hour and by 88% (with only the dose of 50 mg/kg body weight) in the fourth hour (Figure 1). In the first hour, the lowering of blood glucose by the three doses and insulin (1 iu/kg body weight) was similar to that of the normal control mice. Thereafter, the dose of 50 mg/kg body weight reduced blood glucose levels to values significantly lower than those produced by insulin (*p* < 0.05) from the second to the fourth hour, while the dose of 100 mg/kg body weight showed the same effects from the second to the third hour (Table 2).

The lower doses of 25, 35 and 45 mg kg⁻¹ body weight of the same extract lowered the blood sugar levels in a dose dependent manner. This glucose lowering effect was similar to those produced in mice treated with insulin (1 iu/kg body weight) and normal control mice in the first and second hour. Thereafter, from the third hour to the fourth hour, the doses of 35 and 45 mg kg⁻¹ body weight significantly decreased the glucose levels to values lower than those produced by insulin (*p* < 0.05). These glucose levels were significantly lower than those of the normal control mice (*p* < 0.05; Table 3). This decrease in blood sugar was between 58% and 61% in the first hour, 72% and 75% in the second hour, 82% and 88% in the third hour and between 86% and 89% in the fourth hour (Figure 2).
Table 2: Effects of aqueous stem bark extracts of *Warbugia salutaris* on alloxan induced diabetic mice

<table>
<thead>
<tr>
<th>Mice group</th>
<th>Treatment</th>
<th>0 hr</th>
<th>1 hr</th>
<th>2 hrs</th>
<th>3 hrs</th>
<th>4 hrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>Saline</td>
<td>64.3 ± 1.4</td>
<td>58.5 ± 1.9</td>
<td>55.5 ± 1.9</td>
<td>55.3 ± 0.5</td>
<td>57.0 ± 0.7</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>Saline</td>
<td>178.8 ± 6.8*</td>
<td>187.5 ± 9.0*b</td>
<td>195.3 ± 8.6*b</td>
<td>208.5 ± 6.0*b</td>
<td>197.0 ± 22.8*b</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>Insulin</td>
<td>203.0 ± 19.7*</td>
<td>46.3 ± 1.9</td>
<td>54.0 ± 1.5</td>
<td>55.5 ± 3.2</td>
<td>64.3 ± 1.9</td>
</tr>
<tr>
<td>Dose (IU/kg bw)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treated diabetic</td>
<td>Extract</td>
<td>209.8 ± 32.4*</td>
<td>84.5 ± 30.7*a</td>
<td>38.8 ± 2.1**ab</td>
<td>36.5 ± 0.3**ab</td>
<td>26.0 ± 0.0**ab</td>
</tr>
<tr>
<td>Dose (mg/kg bw)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>Extract</td>
<td>173.8 ± 19.9*</td>
<td>43.75 ± 2.3*a</td>
<td>26.0 ± 0.0**ab</td>
<td>26.0 ± 0.0**ab</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>Extract</td>
<td>220.2 ± 22.1*</td>
<td>40.8 ± 2.1*a</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed as means ± SEM for four animals per group. *p <0.05 when compared to normal control treated with saline; *p <0.05 when compared to diabetic control treated with saline and **p <0.05 when compared to diabetic control treated with insulin by student t-test. mg/kg bw stands for mg/kg body weight.
Figure 1: The mean percentage change in blood sugar levels in alloxan induced diabetic mice treated with doses of 50, 100 and 150 mg/kg bw of aqueous stem bark extracts of *Warbugia salutaris*, insulin and saline, and normal mice treated with saline. Values are expressed as % means ± SEM for four animals for each time point. *p <0.05 when compared to normal control treated with saline; ^p <0.05 when compared to diabetic control treated with saline and ^b p <0.05 when compared to diabetic control treated with insulin by student t-test.
Table 3: Effects of aqueous stem bark extracts of *Warbugia salutaris* at lower doses on alloxan induced diabetic mice

<table>
<thead>
<tr>
<th>Mice group</th>
<th>Treatment</th>
<th>0 hr</th>
<th>1 hr</th>
<th>2 hrs</th>
<th>3 hrs</th>
<th>4 hrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>Saline</td>
<td>57.8 ± 3.2</td>
<td>57.0 ± 3.7</td>
<td>57.3 ± 4.2</td>
<td>53.8 ± 1.8</td>
<td>50.0 ± 1.1</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>Saline</td>
<td>211.3 ± 9.0*</td>
<td>214.3 ± 14.4*b</td>
<td>214.3 ± 12.0*b</td>
<td>222.0 ± 13.4*b</td>
<td>226.5 ± 9.8*b</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>Insulin</td>
<td>234.0 ± 21.3*</td>
<td>47.8 ± 1.7</td>
<td>44.8 ± 0.9</td>
<td>46.0 ± 0.4</td>
<td>48.8 ± 0.5</td>
</tr>
<tr>
<td>Dose (IU/kgbw)</td>
<td>Extract</td>
<td>200.3 ± 47.2*</td>
<td>82.5 ± 37.9*a</td>
<td>55.5 ± 15.6*a</td>
<td>36.8 ± 4.5*a</td>
<td>28.5 ± 2.5*ab</td>
</tr>
<tr>
<td></td>
<td>Extract</td>
<td>225.0 ± 24.8*</td>
<td>87.8 ± 17.0*a</td>
<td>57.0 ± 9.4*a</td>
<td>26.0 ± 0.0*ab</td>
<td>26.0 ± 0.0*ab</td>
</tr>
<tr>
<td></td>
<td>Extract</td>
<td>239.8 ± 54.5*</td>
<td>100.0 ± 45.6*a</td>
<td>67.3 ± 25.8*a</td>
<td>28.8 ± 2.8*ab</td>
<td>26.0 ± 0.0*ab</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SEM for four animals per group. *p <0.05 when compared to normal control treated with saline; 
*p <0.05 when compared to diabetic control treated with saline and b*p <0.05 when compared to diabetic control treated with insulin by student t-test. mg/kg bw stands for mg/kg body weight.
Figure 2: The mean percentage change in blood sugar levels in alloxan induced diabetic mice treated with doses of 25, 35 and 45mg/kgbw of aqueous stem bark extracts of *Warbugia salutaris*, insulin, saline and normal mice treated with saline. Values are expressed as % means ± SEM for four animals for each time point.

- *p <0.05* when compared to normal control treated with saline;
- *p <0.05* when compared to diabetic control treated with saline and
- *p <0.05* when compared to diabetic control treated with insulin by student t-test.
The administration of aqueous extracts of the roots of *Terminalia brownii* lowered the sugar levels in a dose dependent manner from the first hour to the fourth hour with the three doses (50, 100 and 150 mg/kg body weight). This decrease in blood sugar was between 36% and 53% in the first hour, 57% and 62% in the second hour, 68% and 69% in the third hour and 74 and 76% in the fourth hour (Figure 3). However, the three doses (50, 100 and 150 mg/kg body weight) lowered blood glucose to values similar to those produced by insulin (1iu/kg body weight) in the third hour. Thereafter, the dose of 150 mg/kg body weight lowered blood glucose levels to values lower than those of the normal control mice in the fourth hour (*p <0.05*) (Table 4).

All the three doses (50, 100 and 150 mg/kg body weight) of aqueous extracts from leaves of *Pentas zanzibarica* lowered the blood sugar from the first hour to the fourth hour in a dose dependent manner (Table 5). This decrease in blood sugar was between 22% and 51% in the first hour, 36% and 69% in the second hour, 44% and 76% in the third hour and 50% and 81% in the fourth hour (Figure 4). These blood sugar levels were similar to those of mice treated with insulin (1iu/kg body weight) in the third hour. The dose of 150 mg kg\(^{-1}\) body weight lowered the blood sugar levels to values lower than those of the normal control mice in the fourth hour (*p <0.05*).

Three doses (50, 100 and 150 mg/kg body weight) from leaf extracts of aqueous *Aloe secundiflora* also lowered the blood sugar from the first hour to the fourth hour though not in a dose dependent manner (Table 6). The decrease in blood sugar was between 33% and 46% in the first hour, 57% and 64% in the second hour, 39% and 71% in the third hour, and 69% and 79% in the fourth hour (Figure 5). The three doses produced similar values of blood glucose levels as those produced in mice treated with insulin (1iu/kg body weight) in the third hour. These values were also similar to those for the normal control mice.
Table 4: Effects of aqueous roots extracts of *Terminalia brownii* on alloxan induced diabetic mice

<table>
<thead>
<tr>
<th>Mice group</th>
<th>Treatment</th>
<th>0 hr</th>
<th>1 hr</th>
<th>2 hrs</th>
<th>3 hrs</th>
<th>4 hrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>Saline</td>
<td>60.8 ± 2.6</td>
<td>57.8 ± 2.5</td>
<td>54.5 ± 1.9</td>
<td>55.0 ± 3.2</td>
<td>54.0 ± 1.9</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>Saline</td>
<td>167.5 ± 17.4*</td>
<td>183.5 ± 14.3*&lt;sup&gt;b&lt;/sup&gt;</td>
<td>197 ± 13.3*&lt;sup&gt;b&lt;/sup&gt;</td>
<td>213.8 ± 8.1*&lt;sup&gt;b&lt;/sup&gt;</td>
<td>227.0 ± 6.1*&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>Insulin</td>
<td>143.3 ± 9.1*</td>
<td>51.25 ± 1.0</td>
<td>49.3 ± 1.6</td>
<td>47 ± 0.4</td>
<td>53.8 ± 2.2</td>
</tr>
<tr>
<td>Dose (1u/kgbw)</td>
<td>Treated diabetic</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>Extract</td>
<td>157.3 ± 8.1*</td>
<td>87.5 ± 1.3*&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>68.0 ± 2.0*&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>49.5 ± 5.1*</td>
<td>40.3 ± 5.8*</td>
</tr>
<tr>
<td>100</td>
<td>Extract</td>
<td>165.3 ± 14.3*</td>
<td>105.3 ± 6.4*&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>68.3 ± 3.4*&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>52.8 ± 1.3*</td>
<td>40.3 ± 1.3*</td>
</tr>
<tr>
<td>150</td>
<td>Extract</td>
<td>163.0 ± 9.0*</td>
<td>77.3 ± 5.3*&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>61.8 ± 7.4*&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>50.0 ± 5.8*</td>
<td>35.8 ± 1.7*&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SEM for four animals per group. *p < 0.05 when compared to normal control treated with saline; *p < 0.05 when compared to diabetic control treated with saline and *p < 0.05 when compared to diabetic control treated with insulin by student t-test. mg/kg bw stands for mg/kg body weight.
Figure 3: The mean percentage change in blood glucose levels in alloxan induced diabetic mice treated with three different doses of aqueous root extracts of *Terminalia brownii*, insulin and saline and normal mice treated with saline. Values are expressed as % means ± SEM for four animals for each time point. *p < 0.05 when compared to normal control treated with saline; ^p < 0.05 when compared to diabetic control treated with saline and ^b p < 0.05 when compared to diabetic control treated with insulin by student t-test.
Table 5: Effects of aqueous leaves extracts of *Pentas zanzibarica* in alloxan induced diabetic mice

<table>
<thead>
<tr>
<th>Mice group</th>
<th>Treatment</th>
<th>Blood glucose levels (mg/dl)</th>
<th>0 hr</th>
<th>1 hr</th>
<th>2 hrs</th>
<th>3 hrs</th>
<th>4 hrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>Saline</td>
<td></td>
<td>65.5 ± 4.8</td>
<td>64.3 ± 3.3</td>
<td>63.3 ± 3.2</td>
<td>62.3 ± 2.9</td>
<td>57.3 ± 1.8</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>Saline</td>
<td></td>
<td>140.0 ± 11.6*</td>
<td>204.0 ± 32.0^ab</td>
<td>228.5 ± 29.3^ab</td>
<td>238.5 ± 29.3^ab</td>
<td>262.8 ± 28.8^ab</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>Insulin</td>
<td></td>
<td>194.0 ± 37.3*</td>
<td>54.0 ± 2.2</td>
<td>50.75 ± 2.4</td>
<td>48.8 ± 1.9</td>
<td>45.5 ± 1.9</td>
</tr>
<tr>
<td>Dose (IU/kgbw)</td>
<td>Extract</td>
<td></td>
<td>116.0 ± 3.9*</td>
<td>91.0 ± 6.4^ab</td>
<td>74.5 ± 7.6^ab</td>
<td>65.5 ± 8.4^a</td>
<td>58.3 ± 6.0^a</td>
</tr>
<tr>
<td></td>
<td>Extract</td>
<td></td>
<td>201.0 ± 27.8*</td>
<td>94.0 ± 4.4^ab</td>
<td>80.3 ± 5.9^ab</td>
<td>66.0 ± 7.3^a</td>
<td>53.3 ± 3.0^a</td>
</tr>
<tr>
<td></td>
<td>Extract</td>
<td></td>
<td>234.3 ± 40.6*</td>
<td>115.0 ± 23.5^ab</td>
<td>73.0 ± 6.5^ab</td>
<td>56.0 ± 4.7^a</td>
<td>45.3 ± 4.5^a</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SEM for four animals per group. '*p <0.05 when compared to normal control treated with saline; "p <0.05 when compared to diabetic control treated with saline and 'p <0.05 when compared to diabetic control treated with insulin by student t-test. mg/kg bw stands for mg/kg body weight.'
Figure 4: The mean percentage change in blood glucose levels in diabetic mice treated with three different doses of aqueous leaf extracts of *Pentas zanzibarica*, insulin and saline and normal mice treated with saline. Values are expressed as % means ± SEM for four animals for each time point. *p <0.05 when compared to normal control treated with saline; *p <0.05 when compared to diabetic control treated with saline and *p <0.05 when compared to diabetic control treated with insulin by student t-test.
Table 6: Effects of aqueous leaves extracts of *Aloe secundiflora* in alloxan induced diabetic mice

<table>
<thead>
<tr>
<th>Mice group</th>
<th>Treatment</th>
<th>0 hr</th>
<th>1 hr</th>
<th>2 hrs</th>
<th>3 hrs</th>
<th>4 hrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>Saline</td>
<td>58.3 ± 4.3</td>
<td>56.3 ± 4.3</td>
<td>53.5 ± 4.0</td>
<td>52.3 ± 3.6</td>
<td>51.5 ± 6.4</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>Saline</td>
<td>147.0 ± 8.5*</td>
<td>162.0 ± 7.4*b</td>
<td>177.5 ± 8.3*b</td>
<td>193.5 ± 7.6*b</td>
<td>203.3 ± 7.9*b</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>Insulin</td>
<td>152.3 ± 11.1*</td>
<td>47.0 ± 3.7</td>
<td>48.0 ± 1.5</td>
<td>40.3 ± 5.2</td>
<td>37.0 ± 4.5</td>
</tr>
<tr>
<td>Dose (IU/kg bw)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>Extract</td>
<td>140.5 ± 11.7*</td>
<td>75.5 ± 2.7*</td>
<td>56.5 ± 2.1*</td>
<td>46.3 ± 0.8*</td>
<td>39.5 ± 1.3*</td>
</tr>
<tr>
<td>100</td>
<td>Extract</td>
<td>131.8 ± 11.7*</td>
<td>88.0 ± 3.1*</td>
<td>56.3 ± 1.4*</td>
<td>45.8 ± 3.4*</td>
<td>40.8 ± 2.8*</td>
</tr>
<tr>
<td>150</td>
<td>Extract</td>
<td>160.0 ± 12.9*</td>
<td>96.0 ± 13.6*</td>
<td>57.3 ± 2.6*</td>
<td>46.3 ± 1.5*</td>
<td>40.3 ± 0.6*</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SEM for four animals per group. *p <0.05 when compared to normal control treated with saline; *p <0.05 when compared to diabetic control treated with saline and *p <0.05 when compared to diabetic control treated with insulin by student t-test. mg/kg bw stands for mg/kg body weight.
Figure 5: The mean percentage change in blood glucose levels in diabetic mice treated with three different doses of aqueous leaf extracts of *Aloe secundiflora*, insulin and saline and normal mice treated with saline. Values are expressed as % means ± SEM for four animals for each time point. *p < 0.05 when compared to normal control treated with saline; *p < 0.05 when compared to diabetic control treated with saline and *p <0.05 when compared to diabetic control treated with insulin by student t-test.
Leaf aqueous extracts of *Olea africana* lowered the blood sugar levels in a dose dependent manner from the first hour to the fourth hour for all the three doses used ie, 50, 100 and 150 mg/kg body weight, respectively. The decrease in the blood sugar was between 24% and 48% in the first hour, 30% and 53% in the second hour, 40% and 61% in the third hour and 46% and 68% in the fourth hour (Figure 6). However, at doses of 100 mg/kg body weight, the extract lowered the blood sugar levels to values similar to those produced by insulin (1 iu/kg body weight) in the fourth hour while at a dose of 150 mg/kg body weight, the blood sugar levels were reduced to values similar to those of mice treated with insulin (1 iu/kg body weight) from the third hour (Table 7).

Blood sugar levels in mice treated with two doses (100 and 150 mg/kg) of aqueous extracts from root tubers of *Rhoicissus tridentata* initially increased up to the first hour, then decreased from the second to the fourth hours. The decrease was dose dependent (Table 8). The initial increase in blood sugar levels was between 27% and 39% in the first hour, a decrease of between 9% and 32% in the second hour, 9% and 15% in the third hour and 11% and 12% in the fourth hour (Figure 7). The 50 mg/kg body weight lowered the blood sugar levels from the first hour to the third hour, and then increased the blood sugar levels in the fourth hour. The decrease in the sugar levels was 20% in the first, 27% in the second hour and 40% in the third hour. The increase in the fourth hour was 33% (Figure 7). Though the three doses lowered the blood sugar levels, from the first to the fourth hour, the values were significantly higher than those of alloxan induced diabetic mice treated with insulin (1 iu/kg body weight) (*p* <0.05) and normal control mice (*p* <0.05)(Table 8).
Table 7: Effects of aqueous leaves extracts of *Olea africana* in alloxan induced diabetic mice

<table>
<thead>
<tr>
<th>Mice group</th>
<th>Treatment</th>
<th>0 hr</th>
<th>1 hr</th>
<th>2 hrs</th>
<th>3 hrs</th>
<th>4 hrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>Saline</td>
<td>64.8 ± 4.0</td>
<td>61.3 ± 4.1</td>
<td>59.3 ± 2.7</td>
<td>56.5 ± 0.7</td>
<td>54.5 ± 1.2</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>Saline</td>
<td>150.0 ± 13.5</td>
<td>172.3 ± 12.7</td>
<td>186.3 ± 12.8</td>
<td>199.3 ± 14.3</td>
<td>216.3 ± 16.3</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>Insulin</td>
<td>129.5 ± 14.5</td>
<td>55.0 ± 1.5</td>
<td>52.3 ± 1.8</td>
<td>50.0 ± 2.7</td>
<td>47.5 ± 3.1</td>
</tr>
<tr>
<td>Treated diabetic</td>
<td>Dose (IU/kg bw)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>122.3 ± 10.4</td>
<td>93.3 ± 2.1</td>
<td>85.5 ± 2.3</td>
<td>72.8 ± 2.5</td>
<td>66.3 ± 1.6</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>117.5 ± 4.4</td>
<td>79.3 ± 1.7</td>
<td>70.8 ± 1.7</td>
<td>61.0 ± 1.5</td>
<td>53.3 ± 1.8</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>141.3 ± 14.4</td>
<td>74.0 ± 1.9</td>
<td>67.0 ± 1.9</td>
<td>55.8 ± 1.7</td>
<td>45.8 ± 1.8</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SEM for four animals per group. *p <0.05 when compared to normal control treated with saline; 'p <0.05 when compared to diabetic control treated with saline and *p <0.05 when compared to diabetic control treated with insulin by student t-test. mg/kg bw stands for mg/kg body weight.
Figure 6: The mean percentage change in blood glucose levels in alloxan induced diabetic mice treated with three different doses of aqueous leaf extracts of *Olea africana*, insulin and saline and normal mice treated with saline. Values are expressed as % means ± SEM for four animals for each time point. *p < 0.05 when compared to normal control treated with saline; *p < 0.05 when compared to diabetic control treated with saline and *p < 0.05 when compared to diabetic control treated with insulin by student t-test.
### Table 8: Effects of aqueous root tuber extracts of *Rhoicissus tridentata* in alloxan induced diabetic mice

<table>
<thead>
<tr>
<th>Mice group</th>
<th>Treatment</th>
<th>0 hr</th>
<th>1 hr</th>
<th>2 hrs</th>
<th>3 hrs</th>
<th>4 hrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>Saline</td>
<td>67.5 ± 5.2</td>
<td>61.3 ± 1.3</td>
<td>57.0 ± 0.4</td>
<td>59.8 ± 0.5</td>
<td>57.8 ± 0.6</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>Saline</td>
<td>138.3 ± 12.0*</td>
<td>162.0 ± 12.6*b</td>
<td>184.5 ± 6.6*b</td>
<td>207.3 ± 9.3*b</td>
<td>218.3 ± 10.8*b</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>Insulin</td>
<td>128.3 ± 11.0*</td>
<td>52.0 ± 1.5</td>
<td>47.0 ± 2.5</td>
<td>44.8 ± 3.2</td>
<td>42.3 ± 3.1</td>
</tr>
<tr>
<td>Dose (IU/kgbw)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treated diabetic</td>
<td>Extract</td>
<td>182.5 ± 27.0*</td>
<td>254.5 ± 27.6<em>b</em>ab</td>
<td>241.0 ± 19.9*ab</td>
<td>210.8 ± 6.5*b</td>
<td>162.5 ± 19.5*ab</td>
</tr>
<tr>
<td>Dose (mg/kgbw)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>Extract</td>
<td>122.5 ± 5.0*</td>
<td>155.5 ± 23.5*b</td>
<td>133.5 ± 14.8*ab</td>
<td>112.0 ± 14.7*ab</td>
<td>107.5 ± 11.6*ab</td>
</tr>
<tr>
<td>100</td>
<td>Extract</td>
<td>170.5 ± 34.1*</td>
<td>136.8 ± 65.2<em>b</em>ab</td>
<td>125.3 ± 42.0*b</td>
<td>102.3 ± 23.8*ab</td>
<td>113.8 ± 41.1*ab</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SEM for four animals per group. *p <0.05 when compared to normal control treated with saline; *p <0.05 when compared to diabetic control treated with saline and *p <0.05 when compared to diabetic control treated with insulin by student t-test. mg/kg bw stands for mg/kg body weight.
Figure 7: The mean percentage change in blood glucose levels in alloxan induced diabetic mice treated with three different doses of aqueous root tuber extracts of *Rhoicissus tridentata*, insulin and saline and normal mice treated with saline. Values are expressed as % means ± SEM for four animals for each time point. *p <0.05 when compared to normal control treated with saline; †p <0.05 when compared to diabetic control treated with saline and ‡p <0.05 when compared to diabetic control treated with insulin by student t-test.
The doses of 100 and 150 mg/kg body weight of aqueous leaf extracts of *Rumex abyssinicus* lowered the blood sugar levels from the first hour to the second hour and later the blood sugar levels rose from the second hour (Table 9). The decrease in blood sugar levels was between 13% and 19% in the first hour and 31% and 43% in the second hour. The increase was between 20% and 23% in the third hour and 12% and 15% in the fourth hour. The dose of 50 mg/kg body weight increased the blood sugar levels in the first hour and then lowered it between the first and third hour. This dose increased the blood sugar levels by 6% in the first hour, and then lowered it by 2% in the second hour. In the third hour it increased the blood sugar levels by between 18% and 4% in the fourth hour. All the three doses produced blood sugar values higher than those produced by alloxan induced diabetic mice treated with insulin (1iu/kg body weight) (*p* <0.05) and those of normal control mice (*p* <0.05) (Table 9).
Table 9: Effects of aqueous leaves extracts of *Rumex abyssinicus* in alloxan induced diabetic mice

<table>
<thead>
<tr>
<th>Mice group</th>
<th>Treatment</th>
<th>0 hr</th>
<th>1 hr</th>
<th>2 hrs</th>
<th>3 hrs</th>
<th>4 hrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>Saline</td>
<td>63.3 ± 4.2</td>
<td>59.5 ± 1.6</td>
<td>55.3 ± 2.0</td>
<td>55.8 ± 3.2</td>
<td>56.0 ± 0.4</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>Saline</td>
<td>177.0 ± 11.2*</td>
<td>187.0 ± 17.9*b</td>
<td>197.8 ± 13.3*b</td>
<td>210.8 ± 8.3*b</td>
<td>228.0 ± 9.9*b</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>Insulin</td>
<td>151.5 ± 8.3*</td>
<td>50.8 ± 2.1</td>
<td>48.8 ± 2.7</td>
<td>45.5 ± 3.2</td>
<td>43.8 ± 3.4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Dose (IU/kgbw)</th>
<th>Treated diabetic</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>Extract</td>
<td>151.5 ± 14.9*</td>
<td>161.0 ± 6.5*b</td>
<td>148.3 ± 8.9*ab</td>
<td>124.5 ± 17.8*ab</td>
<td>145.0 ± 11.4*ab</td>
</tr>
<tr>
<td>100</td>
<td>Extract</td>
<td>139.8 ± 19.0*</td>
<td>113.5 ± 17.1*ab</td>
<td>79.0 ± 7.0*ab</td>
<td>108.0 ± 16.4*ab</td>
<td>123.3 ± 11.4*ab</td>
</tr>
<tr>
<td>150</td>
<td>Extract</td>
<td>130.5 ± 12.1*</td>
<td>113.3 ± 10.2*ab</td>
<td>90.5 ± 3.9*ab</td>
<td>104.3 ± 9.6*ab</td>
<td>110.8 ± 11.5*ab</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SEM for four animals per group. *p <0.05 when compared to normal control treated with saline; *p <0.05 when compared to diabetic control treated with saline and *p <0.05 when compared to diabetic control treated with insulin by student t-test. mg/kg bw stands for mg/kg body weight.
Figure 8: The mean percentage change in blood glucose levels in alloxan induced diabetic mice treated with three different doses of aqueous root tuber extracts of *Rumex abyssinicus*, insulin and saline and normal mice treated with saline. Values are expressed as % means ± SEM for four animals for each time point. *p <0.05 when compared to normal control treated with saline; *p <0.05 when compared to diabetic control treated with saline and *p <0.05 when compared to diabetic control treated with insulin by student t-test.
The aqueous plant extracts of *Warbugia salutaris*, *Terminalia brownii*, *Pentas zanzibarica*, *Aloe secundiflora*, *Olea africana* and *Rhoicissus tridentata* that demonstrated blood glucose lowering effect in alloxan induced diabetic mice were further subjected to elemental analysis, phytochemical screening and toxicity tests.

3.2 Elemental Analysis of the Aqueous Plant Extracts

Table 10 shows that *Aloe secundiflora* contained Cr, Cu, Fe, Pb, Mg, Mo and Zn. *Olea africana* contained Mn, Fe, Cu, Zn, Mo, Pb, Cr and Mg while *Pentas zanzibarica* contained Cr, Cu, Fe, Pb, Mg and Zn. *Terminalia brownii* had Cr, Fe, Pb, Mg and Zn and *Warbugia salutaris* contained Cr, Cu, Fe, Pb, Ni, Mg, Mo, Sr and Zn. *Rhoicissus tridentata*, contained all the analysed elements apart from Ni, Sr and V. All the plants extracts did not contain detectable quantities of V while Sr was only found in *Warbugia salutaris*. The only plant extract that contained Ni was *Warbugia salutaris*. These elements were in different quantities (Table 10).

3.3 Phytochemical Screening of the Aqueous Plant Extracts

Table 11 shows the class of compounds present in the aqueous plant extracts of *Aloe secundiflora*, *Olea africana*, *Pentas zanzibarica*, *Terminalia brownii*, *Warbugia salutaris*, *Rhoicissus tridentata* and *Rumex abyssinicus*. The results show that the extracts from *Aloe secundiflora* contained flavanoids (in trace amounts) and anthraquinones. *Olea africana* extracts contained alkaloids, terpenoids, flavanoids and anthraquinones while those of *Pentas zanzibarica* contained sterols, saponins, flavanoids and tannins. *Terminalia brownii* extracts contained saponins, flavanoids and tannins. *Warbugia salutaris* extracts contained terpenoids, saponins and tannins and those of *Rhoicissus tridentata* contained alkaloids,
saponins, tannins and trace amounts of sterols. These classes of compounds were in different quantities (Table 11).
Table 10: Elemental Content of Plant Extracts

<table>
<thead>
<tr>
<th>Element</th>
<th>A. secundiflora</th>
<th>O. africana</th>
<th>P. zanzibarica</th>
<th>T. brownii</th>
<th>W. salutaris</th>
<th>R. tridentata</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cr</td>
<td>78.2±6.1</td>
<td>90.4±18.3</td>
<td>114.7±18.3</td>
<td>84.3±0.0</td>
<td>108.6±12.2</td>
<td>108.6±24.3</td>
</tr>
<tr>
<td>Cu</td>
<td>10.2±0.1</td>
<td>8.2±1.2</td>
<td>32.0±5.0</td>
<td>&lt;5</td>
<td>24.8±3.1</td>
<td>12.0±2.0</td>
</tr>
<tr>
<td>Fe</td>
<td>238.0±19.0</td>
<td>133.0±11.0</td>
<td>790.0±133.0</td>
<td>107.0±11.0</td>
<td>204.0±31.0</td>
<td>76.0±8.0</td>
</tr>
<tr>
<td>Pb</td>
<td>8.9±0.5</td>
<td>9.7±0.6</td>
<td>16.0±1.0</td>
<td>10.1±0.8</td>
<td>7.2±0.9</td>
<td>8.0±1.0</td>
</tr>
<tr>
<td>Ni</td>
<td>&lt;5</td>
<td>&lt;5</td>
<td>&lt;5</td>
<td>&lt;5</td>
<td>14.1±1.7</td>
<td>&lt;5</td>
</tr>
<tr>
<td>Mg</td>
<td>4194.9±158.2</td>
<td>3702.2±47.6</td>
<td>3572.5±48.7</td>
<td>4705.6±1.5</td>
<td>4350.9±0.8</td>
<td>3507.2±22.5</td>
</tr>
<tr>
<td>Mn</td>
<td>&lt;5</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
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<td>&lt;1</td>
</tr>
<tr>
<td>Mo</td>
<td>4.3±0.6</td>
<td>3.4±0.1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>2.0±0.2</td>
<td>2.1±0.4</td>
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<tr>
<td>Sr</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>2.0±0.3</td>
<td>&lt;1</td>
</tr>
<tr>
<td>V</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
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<td>&lt;1</td>
</tr>
<tr>
<td>Zn</td>
<td>499.0±38.0</td>
<td>105.0±7.0</td>
<td>94.0±7.0</td>
<td>41.7±6.2</td>
<td>48.2±5.3</td>
<td>43.0±1.0</td>
</tr>
</tbody>
</table>

*Elements analysed using AAS; <1 and <5 is below the limit of detection of EDXRF; <1* is below the limit of detection of AAS
Table 11: Classes of Compounds in the Plant Extracts

<table>
<thead>
<tr>
<th>Botanical names</th>
<th>Plant part</th>
<th>Alkaloids</th>
<th>Sterols</th>
<th>Terpenoids</th>
<th>Saponins</th>
<th>Flavanoids</th>
<th>Tannins</th>
<th>Anthraquinones</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aloe secundiflora</td>
<td>Leaves</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Olea africana</td>
<td>Leaves</td>
<td>++</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Pentas zanzibarica</td>
<td>Leaves</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Terminalia brownii</td>
<td>Roots</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Warbugia salutaris</td>
<td>Stem bark</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>++</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Rhoicissus tridentata</td>
<td>Root tubers</td>
<td>+</td>
<td>-+</td>
<td>-</td>
<td>++</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

**Key**

++ highly positive  + positive  -+ trace  - negative
3.4 Preliminary Histological Assessment of Toxicity in vivo

Histologically, various tissue sections from the mice treated with *Olea africana* extract did not show any pathological lesions. However, when the mice were treated with the dose of 450mg/kg body weight of aqueous stem bark extract of *Warbugia salutaris* for 30 days, sections of spleens showed follicular haemorrhage (Plate 2) and in the liver, the hepatocytes were vacuolated (Plate 4). The liver of the mice treated with aqueous leaf extract of *Pentas zanzibarica* (450mg/kg body weight) for 30 days showed vascular congestion (Plate 6). The mice that were treated with a dose of 450mg/kg body weight of aqueous root extract of *Rhoicissus tridentata* for 30 days, the spleen showed a pronounced lymphoid depopulation (Plate 7), severe tubular degeneration in the kidney (Plate 8) and in the heart, the myofibrils were swollen and discontinuous (Plate 10). Spleens of mice injected with *Aloe secundiflora* and *Terminalia brownii* showed mild lymphoid depopulation, respectively (Plate 11 and 12).
Plate 1: Histological section of the spleen from a normal mouse treated with physiological saline (0.1ml/day) for 30 days. ×100

Plate 2: Histological section of a spleen from a mouse treated with an aqueous stem bark extracts of *Warbugia salutaris* (450mg/kg body weight/day) for 30 days. Haemorrhagic foci (arrow) are observable. ×100
Plate 3: Histological section of a liver from a normal mouse treated with physiological saline (0.1ml/day) for 30 days. ×100

Plate 4: Histological section of a liver from a mouse treated with an aqueous stem bark extracts of *Warbugia salutaris* (450 mg/kg body weight/day) for 30 days. Vacuolated hepatocytes (mild hepatotoxicity) (arrows) are evident. ×100
Plate 5: Histological section of a kidney from a normal mouse treated with physiological saline (0.1 ml/day) for 30 days. ×400

Plate 6: Histological section of a kidney from a mouse treated with an aqueous leaves extracts of Pentas zanzibarica (450 mg/kg body weight/day) for 30 days. Vascular congestion (arrows) is observable. ×400
Plate 7: Histological section of a spleen from a mouse treated with an aqueous root extract of *Rhoicissus tridentata* (450 mg/kg body weight/day) for 30 days. Wide cell-to-cell boundaries (arrows) are observable. ×100

Plate 8: Histological section of a kidney from a mouse treated with an aqueous root extract of *Rhoicissus tridentata* (450 mg/kg body weight/day) for 30 days. Severe tubular degeneration (arrows) is evident. ×400
Plate 9: Histological section of a heart from a normal mouse treated with physiological saline (0.1ml/day) for 30 days. ×400

Plate 10: Histological section of a heart from a mouse treated with an aqueous root extract of *Rhoicissus tridentata* (450 mg/kg body weight/day) for 30 days. Swollen discontinuous myofibrils (arrows) are observable. ×400
Plate 11: Histological section of a spleen from a mouse treated with an aqueous leaf extract of *Aloe secundiflora* (450 mg/kg body weight/day) for 30 days. Wide cell-to-cell boundaries (arrows) are observable. x100

Plate 12: Histological section of a spleen from a mouse treated with an aqueous leaf extract of *Terminalia brownii* (450 mg/kg body weight/day) for 30 days. Wide cell-to-cell boundaries (arrows) are observable. x100
Chapter Four
Discussion, Conclusion and Recommendations

4.1 Discussion

That mice injected intraperitoneally with 150mg/kg body weight alloxan and left for three to four days had a three to four fold increase in blood glucose (100 mg/dL to 200 mg/dL) relative to the normal control mice, could be explained by the specific necrosis of pancreatic islets. This occurs after alloxan destroys and reduces the β cells via formation of reactive oxygen species like nitric oxide (Szkudelski 2001). The glucose lowering effect of aqueous extracts of *Warbugia salutaris*, *Terminalia brownii*, *Pentas zanzibarica*, *Aloe secundiflora*, *Olea africana* and *Rhoicissus tridentata* in alloxan induced diabetic mice indicates that these plants contain hypoglycaemic constituents. That the aqueous extracts of *Warbugia salutaris*, *Olea africana*, *Terminalia brownii* and *Pentas zanzibarica* lowered the blood sugar levels in a dose dependent manner indicates that the extracts might have been absorbed through the cell lipid membranes through facilitated diffusion. In this, the ions might have been transported in the direction of its electrochemical gradient. This trend is in agreement in with expectations seen in administration of higher concentration of a drug. Similar studies on anti-diabetic activity of *Allium cepa* in diabetic animals demonstrated a reduction in blood sugar in a dose dependent manner (Sharma *et al.* 1977). The lowering effect of blood sugar levels by *Aloe secundiflora* in the same manner regardless of the concentration of the drug 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 with the rest of extract being excreted. This study concurs with other findings of different *Aloe* species (*Aloe vera*) which also demonstrated anti-diabetic effects (Okyar *et al.* 2001). That *Rhoicissus tridentata* showed a lower effect in lowering blood sugar than insulin suggests the possibility of islets repair or mimicry of insulin action by
elements from the extract. Death at higher doses of *Warbugia salutaris* (50, 100 and 150 mg/kg body weight), in alloxan induced diabetic mice could have been caused by acute hypoglycaemia just like the administration of high concentration of other drugs (Raing *et al.* 2000). This is supported by the observation that at lower doses (25, 35 and 45 mg kg⁻¹ body weight) of the extract, no death occurred.

The lowering of blood sugar level by these extracts could also be attributed to the presence of inorganic mineral elements like chromium, magnesium, molybdenum, iron and zinc. Magnesium acts as a cofactor to several enzymes like kinase, which participate in energy production processes. Chromium is important for fat metabolism, enzyme activation and the regulation of cholesterol. Chromium inhibits the synthesis of new fat from carbohydrates, thus freeing the mitochondria to burn already stored fat (Anderson *et al.* 1997). Studies done by Li *et al.* (1995) have showed that molybdenum stimulates glycolysis and accelerates glycogen degradation in the hepatocytes and also increases insulin receptor autophosphorylation and phosphorylation of its substrate and augmented glucose transport, oxidation and lipogenesis in adipocytes. Zinc influences glyceraldehyde-3-phosphate dehydrogenase, the enzyme involved in glycolysis while iron is found in the portion of the cell involved in energy production and acts as a cofactor for several enzymes. Nevertheless, the mechanisms of action for zinc in energy production is still obscure (Jose Manuel *et al.* 2002). In addition to these elements, aqueous extract of *Pentas zanzibarica, Olea africana* and *Rhoicissus tridentata* were found to possess manganese. Manganese has been reported by Friedman (1987) to be both an activator and a constituent of several enzymes like kinases and enzymes of oxidative phosphorylation.

The blood glucose lowering effect of these plant extracts may also be attributed to the presence alkaloids, flavanoids and tannins, which have been associated with hypoglycemic activity. Anti-diabetic effects of *Rhoicissus tridentata* and *Olea africana* could have been due
to the presence of alkaloids. Alkaloids are known to suppress hyperglycemia. Elliott et al. (2000) reported that alkaloids such as alkaloid 1-ephedrine promotes the regeneration of pancreas islets following atrophy, restores the secretion of insulin, and thus correct hyperglycemia. Flavonoids present in Terminalia brownii, Pentas zanzibarica, Aloe secundiflora and Olea africana could also have contributed to hypoglycaemic activities. As reported by Glauce et al. (2004), flavonoids like myricetin have insulinomimetic properties and stimulates lipogenesis and glucose transport in the adipocytes hence lowering blood sugar. Similar studies done on Pterocarpus marsupium found epicatechin and catechin flavanoids having anti-diabetic properties (Subramanian 1981). The aqueous extracts of Warbugia salutaris, Terminalia brownii, Pentas zanzibarica and Rhoicissus tridentata also possessed tannins that probably might have caused hypoglycaemic activities. Tannins like epigallo-catechin-3-gallate exhibit anti-diabetic activity as has been reported by Broadhurst et al. (2000).

That root tubers of aqueous extracts of Rhoicissus tridentata which showed a lower effect in reducing the blood sugar as compared to insulin, suggests the presence of serum insulin due to the recovery of the pancrease or mimicry of insulin action probably by elements from the extract hence correcting hyperglycemia. Like alkaloids, zinc could be involved in the regeneration of the destroyed pancreas islets by the alloxan. Zinc has been reported by Ho et al. (2001) to regenerate the destroyed endocrine pancrease. This correlates to a study done on Gymnema sylvestre leaf extracts which were found to regenerate the islets of streptozotocin-induced diabetic rats (Shanmugasundaram et al. 1990).

Even though these classes of compounds are postulated to contribute to hypoglycaemic activities, preliminary studies done on the indigenous hypoglycaemic herbs have shown that inorganic mineral elements pronounce more hypoglycaemic potentials than their corresponding organic parts (Kar et al. 1999). Therefore, the elements in these extracts
could be hypoglycaemic by depressing glucose synthesis; on one hand through depressing the key gluconeogenic enzymes like glucose-6-phosphate phosphatase and fructose-1,6-biphosphatase and on the other hand, by enhancing glucose oxidation by the shunt pathway through activation of glucose 6 phosphate dehydrogenase (G6PDH). These extracts may also contain insulin-like polypeptides, which mimic insulin action. Similar studies done on hypoglycaemic activity of *Coccinia indica* and *Momordica charantia* were found to possess these principles (Shibib *et al.* 1993).

In several studies, mice are used as models for human beings. Therefore, the recommended daily allowance (RDA) for a mouse for each element can be postulated from the known RDA for human beings. In this study, it can therefore be postulated that in the histological toxicity assay, the amounts of individual elements injected to a mouse per day were less than their respective recommended daily allowances. In this regard, only the nickel in aqueous stem bark extract from *Warbugia salutaris* could have caused the toxic effects in the tissues. Even though chromium given to mice in all extracts was higher than the RDA’s, it might not have contributed to toxicity since no toxic effects have ever been attributed to it in literature. In the other plants extracts, the histological toxic effects might have then been caused by the different classes of compounds present (Appendix V). Nevertheless, further studies should be done to determine the RDA of trace elements for mice.

That treatment of the spleen with aqueous extracts of *Warbugia salutaris* showed some degree of hemorrhage while that with aqueous extracts of *Rhoicissus tridentata* showed pronounced lymphoid depopulation in the spleen could be due to the presence of cytotoxic saponins which cause hemorrhage to tissues and also lymphopoietic cell destruction, respectively. Similar toxicity pattern were observed from evaluation of histopathological changes due to saponins in mice (Diwan *et al.* 2000). Anthraquinones found in *Aloe*
secundiflora may have also caused lymphopoietic cell destruction. Anthraquinones are known to be cytotoxic in mice (Westendorf et al. 1988).

Death of mice occurred after administration of Warbugia salutaris extract, and necrosis of the liver (mild hepatotoxicity) was observed. This could be attributed to the presence of terpenoids and nickel. In the hepatocytes, terpenoids and nickel might have been exposed to phase I and phase II reactions involved in drug metabolism and produced more reactive species such as those formed by P450 enzymes causing hepatotoxicity. Terpenes like beta-myrcene are toxic to the stomach, liver and highly irritant to the peritoneum. Deaths after intraperitoneal injection in rats and mice possibly due to drug induced chemical peritonitis have been observed (Paumgartten et al. 1990). Nickel sulphate has been shown to be highly toxic especially to seminal vesicles and other tissues in mice (Pandey and Singh 2001; Suzan et al. 1999).

The presence of vacuolated and congested hepatocytes is an indication of hepatotoxicity after treatment with aqueous extract of Pentas zanzibarica and Rhoicissus tridentata respectively. This could be due to the presence saponins and tannins. In one study, saponins led to haemorrhage in many liver lobules, congestion of central veins and sinusoids that led to necrosis of liver cells (Diwan 2000). Similarly, tannins have been shown to cause severe central necrosis of the liver (Chung et al. 1998). Alkaloids present in Rhoicissus tridentata could also have contributed to the observed toxicity. Alkaloids such as pyrrolizidine alkaloids (PAS), which are metabolized in the liver by means of hydrolysis, N-oxidation and demethylation, have also been shown to have the same toxic effects. Metabolites derived from N-oxidation process mainly affect the liver leading to intense cellular alterations known as megalocytosis (Zeinsteger et al. 2003). The myocardial degeneration (cardiotoxicity) observed after treatment with Rhoicissus tridentata could be as a result of saponins present in the plant extract.
That the blood capillaries in the kidneys were prominently enlarged (moderately congested) and severe tubular degeneration evident after treatment with aqueous extract of *Pentas zanzibarica* and *Rhoicissus tridentata*, respectively, is an indication of severe nephropathy. This could be attributed to the presence of saponins and sterols in the plant extracts. Probably, these saponins and sterols are steroidal compounds, which like other compounds like serotonin, contracts vascular system especially in the kidney and lungs hence cause prominent enlargement of blood capillaries.

4.2 Conclusion

In conclusion, 85.7% (6/7) of the seven plant species used traditionally in the practice of herbal medicine had antidiabetic activities and 16.7% of the plant species with antidiabetic activity were not toxic (*Olea africana*) while the other 83.3% were toxic (*Warbugia salutaris, Pentas zanzibarica, Terminalia brownii, Aloe secundiflora* and *Rhoicissus tridentata*). This would account for their ethno-pharmacological uses as anti-diabetics in herbal medical practice. The toxic anti-diabetic plants are still used in the traditional medicine and have not been reported to cause toxicity because they are used as combination of many plants, which may result in the reduction of toxicity and an increase in the therapeutic index.

4.3 Recommendations

- The plants that exhibited anti-diabetic activities in aqueous extracts should also be subjected to organic solvent extraction and compare activities of both aqueous and organic fractions.
- It would also be necessary to study different parts of the same plants such as root bark, stem bark, leaves to establish the regions with the highest concentrations of anti-diabetic activity.
• The hypoglycaemic extracts should be subjected to different dose titrations to determine their in vivo effective and lethal doses. The concentrations and dosage of the active plant principles can be formulated to improve their effectiveness and this knowledge availed to the traditional medical practitioners.

• The isolation of compounds present in the hypoglycaemic extracts should be undertaken, and subjected to bioassays, to investigate whether the individual isolated compounds retain or lose their activity or are more active than their parent crude extracts.

• A study should be undertaken to establish whether there are seasonal variations in the hypoglycaemic activity of these plant extracts.

• The hypoglycaemic effects of the anti-diabetic plants should also be investigated to assess their antidiabetic activity when administered via the oral route.

• Determine the frequency of dosage of the active extracts.

• The mechanism of action of these hypoglycaemic plant extracts should also be established using the normal mice.

• Histopathological, biochemical and immunological toxicity of these extracts in all body tissues and/or organs should be investigated.

• The antidiabetic plant extracts should be developed into capsules or tablets to increase their useful period and avoid the development of toxic fermentation products. This will avail crude drugs that are cheaper and affordable to the local population as has been done in East Asia using (Panax quinquefolius) Panax ginseng.

• Conservation and commercial farming of the antidiabetic plants should also be promoted.

• Assess the effectiveness of some specific trace elements in lowering blood glucose.

• Studies should be undertaken to determine the RDA of trace elements of mice.
References


Shibib BA, Khan LA, Rahman R (1993). Hypoglycaemic activity of *Coccinia indica* and *Momordica charantia* in diabetic rats; depression of the hepatic gluconeogenic enzymes glucose-6-phosphatase and fructose-1,6-bisphosphatase and elevation of both liver and red-


Appendix I

Instrumental conditions for AAS

The conditions for optimum settings for the analysis of each of the elements by Atomic Absorption Spectrophotometer (AAS) are summarized in the table below.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Chromium</th>
<th>Magnesium</th>
<th>Vanadium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wavelength (nm)</td>
<td>357.9</td>
<td>285.2</td>
<td>318.4</td>
</tr>
<tr>
<td>Slit width (nm)</td>
<td>0.7</td>
<td>0.7</td>
<td>0.2</td>
</tr>
<tr>
<td>Lamp current (mA)</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Flow rate (l/min)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Air</td>
<td>7.0</td>
<td>7.0</td>
<td>7.0</td>
</tr>
<tr>
<td>Acetylene</td>
<td>1.8</td>
<td>1.8</td>
<td>1.8</td>
</tr>
</tbody>
</table>

Optimal conditions were achieved by following the procedure outlined in the instruction manual, which require that the instrument be allowed to warm up for 15 minutes. Then maximum and minimum scale readings were set using the most concentrated standard and blank reagent, respectively.
Appendix II

A graph of standard curve for Vanadium

<table>
<thead>
<tr>
<th>Concentration (ppm)</th>
<th>1</th>
<th>5</th>
<th>10</th>
<th>15</th>
<th>20</th>
<th>25</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absorbance</td>
<td>0.001</td>
<td>0.019</td>
<td>0.04</td>
<td>0.054</td>
<td>0.077</td>
<td>0.093</td>
</tr>
</tbody>
</table>

Standard Curve for Vanadium

\[ y = 0.0185x - 0.0175 \]
Appendix III

A graph of standard curve for Chromium

<table>
<thead>
<tr>
<th>Concentration (ppm)</th>
<th>1</th>
<th>5</th>
<th>10</th>
<th>15</th>
<th>20</th>
<th>25</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absorbance</td>
<td>0.0015</td>
<td>0.018</td>
<td>0.0385</td>
<td>0.06</td>
<td>0.0795</td>
<td>0.1</td>
</tr>
</tbody>
</table>

Standard Curve for Chromium

\[ y = 0.02x - 0.0203 \]

Absorbance trendline
Appendix IV

A graph of standard curve for Magnesium

<table>
<thead>
<tr>
<th>Concentration (ppm)</th>
<th>1</th>
<th>5</th>
<th>10</th>
<th>15</th>
<th>20</th>
<th>25</th>
<th>30</th>
<th>50</th>
<th>70</th>
<th>90</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absorbance</td>
<td>0.08</td>
<td>0.288</td>
<td>0.49</td>
<td>0.629</td>
<td>0.809</td>
<td>0.877</td>
<td>1.106</td>
<td>1.772</td>
<td>2.439</td>
<td>3.105</td>
<td>3.438</td>
</tr>
</tbody>
</table>

Standard Curve for Magnesium

y = 0.2568x - 0.4082
Appendix V

Table 12: Quantity of elements in plant extracts administered to a mouse per day (µg/day) and the Recommended daily allowance (RDA) of the elements for a mouse

<table>
<thead>
<tr>
<th>Element</th>
<th>(A.\ secundiflora)</th>
<th>(O.\ africana)</th>
<th>(P.\ zanzibarica)</th>
<th>(T.\ brownii)</th>
<th>(W.\ salutaris)</th>
<th>(R.\ tridentata)</th>
<th>RDA for a mouse/day (µg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cr</td>
<td>0.63</td>
<td>0.72</td>
<td>0.92</td>
<td>0.67</td>
<td>0.87</td>
<td>0.87</td>
<td>0.01</td>
</tr>
<tr>
<td>Cu</td>
<td>0.08</td>
<td>0.07</td>
<td>0.26</td>
<td>0.04</td>
<td>0.2</td>
<td>0.1</td>
<td>0.32</td>
</tr>
<tr>
<td>Fe</td>
<td>1.9</td>
<td>1.06</td>
<td>6.32</td>
<td>0.86</td>
<td>1.63</td>
<td>0.61</td>
<td>10.71</td>
</tr>
<tr>
<td>Pb</td>
<td>0.07</td>
<td>0.08</td>
<td>0.13</td>
<td>0.08</td>
<td>0.06</td>
<td>0.06</td>
<td>None</td>
</tr>
<tr>
<td>Ni</td>
<td>&lt; 0.04</td>
<td>&lt; 0.04</td>
<td>&lt; 0.04</td>
<td>&lt; 0.04</td>
<td>0.11</td>
<td>&lt; 0.04</td>
<td>None</td>
</tr>
<tr>
<td>Mg</td>
<td>35.55</td>
<td>29.62</td>
<td>28.58</td>
<td>37.64</td>
<td>34.8</td>
<td>28.06</td>
<td>150</td>
</tr>
<tr>
<td>Mn</td>
<td>&lt; 0.04</td>
<td>1.2</td>
<td>0.14</td>
<td>&lt; 0.04</td>
<td>&lt; 0.04</td>
<td>0.09</td>
<td>0.82</td>
</tr>
<tr>
<td>Mo</td>
<td>0.03</td>
<td>0.03</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
<td>0.02</td>
<td>0.02</td>
<td>0.021</td>
</tr>
<tr>
<td>Sr</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
<td>0.02</td>
<td>&lt; 0.01</td>
<td>None</td>
</tr>
<tr>
<td>V</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
<td>0.64</td>
</tr>
<tr>
<td>Zn</td>
<td>4</td>
<td>0.84</td>
<td>0.75</td>
<td>0.33</td>
<td>0.36</td>
<td>0.34</td>
<td>6.79</td>
</tr>
</tbody>
</table>