IN VIVO HYPOGLYCEMIC ACTIVITY AND TOXICITY OF FIVE SELECTED MEDICINAL PLANTS TRADITIONALLY USED TO MANAGE DIABETES MELLITUS IN MACHAKOS COUNTY, KENYA

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A Thesis Submitted in Partial Fulfillment of the Requirements for the Award of the Degree of Masters of Science (Medical Biochemistry) in the School of Pure and Applied Science of Kenyatta University

OCTOBER 2015
DECLARATION

I, Abdirahman Yusuf Ahmed, duly declare that this thesis is my original work and has not been presented for a degree in any other university or for any other award.

Abdirahman Yusuf Ahmed (BSc. Hons)

Signature Abdirahman Date 22/10/2015

We hereby confirm that the candidate carried out the work reported in this thesis under our supervision.

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Signature Date 23/10/2015
DEDICATION
I hereby dedicate this thesis to my wife Mrs Zainab Mohamud Jumale, to whom I am very grateful for her support and encouragement.
ACKNOWLEDGEMENT
First and above all, I thank Allah (God), the Almighty, for having made everything possible by giving me strength and courage to do this work.

I want to express my deep thanks and appreciations to my supervisors Prof Eliud N M Njagi, Prof Joseph J N Ngeranwa and Prof Peter K Gathumbi for their support and tremendous guidance from the beginning of this work to its conclusion.

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Insulin therapy
Oral hypoglycemic drugs
Biguanides
Sulphonylureas
Glinides
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Thiazolidinediones
Incretin-based therapies
Herbal management of diabetes mellitus

Allium cepa and Allium sativum
Nigella sativa
Aloe vera
Azadirachta indica
Gymnema sylvestre
Momordica charantia
Plants of this study
Kleinia squarrosa (locally known as Mung'endya nthenge)
Acacia nilotica (locally known as Musemei)
Aloe secundiflora (locally known as Kiluma)
Zanha africana (locally known as Mukolekya)
Fuerstia africana (Locally known as kalak"u)

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<td>AACE</td>
<td>American Association of Clinical Endocrinology</td>
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<td>AAS</td>
<td>Atomic Absorption Spectrometry</td>
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<td>ABCC8</td>
<td>ATP Binding Cassette, Subfamily C, Member 8</td>
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<td>ADA</td>
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<td>AIDS</td>
<td>Acquired Immune Deficiency Syndrome</td>
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<td>ALP</td>
<td>Alkaline Phosphatase</td>
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<td>Alanine Transaminase</td>
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<td>APC</td>
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<td>CALPN10</td>
<td>Calpain 10</td>
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<td>cAMP</td>
<td>Cyclic Adenosine Mono Phosphate</td>
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<td>Creatine Kinase</td>
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<td>CNP</td>
<td>2-Chloro-4-Nitrophenol</td>
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<td>CNPG3</td>
<td>2-Chloro-4-Nitrophenyl-A-D-Maltotrioside (CNPG₃)</td>
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<td>Central Nerve System</td>
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<td>Diabetes Keto Acidosis</td>
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<td>Deoxyribonucleic Acid</td>
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<td>DPP4</td>
<td>Dipeptidyl Peptidase 4</td>
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<td>EDTA</td>
<td>Ethylene Diamine Tetra-acetic Acid</td>
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<td>HbA1c</td>
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<td>Human Leukocyte Antigen</td>
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<td>IDDM</td>
<td>Insulin Dependent Diabetes Mellitus</td>
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<td>IDF</td>
<td>International Diabetes Federation</td>
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<td>Abbreviation</td>
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<tr>
<td>IGT</td>
<td>Impaired Glucose Tolerance</td>
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<td>IL2RA</td>
<td>Interleukin 2 Receptor, Alpha</td>
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<td>KCNJ11</td>
<td>The Potassium Channel, Inwardly Rectifying Subfamily J, Member 11</td>
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<td>LDH</td>
<td>Lactate Dehydrogenase</td>
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<td>MCH</td>
<td>Mean Corpuscular Hemoglobin</td>
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<td>MCHC</td>
<td>Mean Corpuscular Hemoglobin Concentration</td>
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<td>MCV</td>
<td>Mean Corpuscular Volume</td>
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<td>MDH</td>
<td>Malate Dehydrogenase</td>
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<td>MHC</td>
<td>Major Histocompatibility Complex</td>
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<td>NAD</td>
<td>Nicotinamide Adenine Dinucleotide</td>
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<td>NDIC</td>
<td>National Diabetes Information Clearinghouse</td>
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<td>NIDDM</td>
<td>Non-Insulin Dependent Diabetes Mellitus</td>
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<td>NPDR</td>
<td>Non-Proliferative Diabetic Retinopathy</td>
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<td>PCV</td>
<td>Packed Cell Volume</td>
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<td>Vascular Endothelial Growth Factor (VEGF)</td>
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<tr>
<td>VLDL</td>
<td>Very Low Density Lipoproteins</td>
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<td>WBC</td>
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<td>α-AMYL</td>
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ABSTRACT

Diabetes mellitus is a chronic disorder with increasing rates of incidence and mortality. Antidiabetic drugs are either expensive or unavailable or they have side effects which can lead to other complication to the patients. *Kleinia squarrosa*, *Acacia nilotica*, *Zanha Africana*, *Aloe secundiflora*, and *Fuerstia Africana* have been used traditionally to manage several diseases including diabetes, however, their efficacy and toxicity is not well evaluated. The aim of this study was to determine the *in vivo* hypoglycemic activity and toxicity of aqueous extracts of these plants in male swiss white albino mice. Hypoglycemic activity was screened in alloxan induced diabetic mice using oral and intraperitoneal routes. The toxicity of these aqueous extracts was studied in mice that were orally and intraperitoneally administered with 1g/kg body weight daily for 28 days by recording changes in body and organ weights, hematological and biochemical parameters and histology. Composition of trace elements in the plant extracts were estimated using total reflection X-ray fluorescence system and atomic absorption spectrometry. Phytochemical analysis was assessed using standard procedures. Stem bark extract of *K. squarrosa*, *A. nilotica*, and *A. secundiflora*, and leaf extracts of *Z. africana* administered at 50, 100, 200, 300 mg/kg body weight showed hypoglycemic activity with the intraperitoneal route being more effective than the oral route except for *A. secundiflora*. Oral administration 1g/kg body weight of the stem bark extract of *K. squarrosa* and leaf extract of *Z. africana* and intraperitoneal administration of all plant extracts significantly reduced the body weight gain. Orally, the same dose of *K. squarrosa* reduced the percent organ to body weight of the liver. The intraperitoneal administration of some extracts increased the percent organ to body weight of the liver, brain, kidneys and lungs while that of the testis was reduced by others. Oral or intraperitoneal administration of the same dose of each the studied plant extracts significantly affected on or more of measured hematological parameters. Oral and intraperitoneal administration of the same dose of the aqueous plant extracts significantly altered the biochemical parameters. Intraperitoneal administration of the same dose of *K. squarrosa* to mice caused significant lesions to the kidney, liver and spleen. All the five aqueous plants extracts studied contained tannins, total phenols, flavonoids, saponins and alkaloids at varying levels. Sodium, Chlorine, Potassium, Calcium, Titanium, Vanadium, Chromium, Manganese, Iron, Copper, Zinc, Arsenic and Cadmium were present in all the five plant extracts at levels below the recommended daily allowance. Magnesium, Nickel and Lead were present in four plant extracts. The observed hypoglycemic activity could be associated with phytochemicals and mineral elements present in the aqueous extracts of the studied plants. Toxic effects of the studied plants at a dose of 1g/kg body weight may be due to their phytochemical components together with the presence of heavy metals like arsenic, lead and cadmium in the plants extracts. This study recommends continued use of these plants as herbal medicine except *Kleinia squarrosa* which was strongly toxic at 1 g/kg body weight.
CHAPTER ONE
INTRODUCTION

1.1 Background information

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels (American Diabetes Association “ADA”, 2012a). The basic effect of insulin lack or insulin resistance on glucose metabolism is inefficient glucose uptake and utilization by most cells of the body except those of the brain. As a result blood glucose concentration increases, cell utilization of glucose falls and utilization of fat and protein increases (Guyton & Hall, 2000).

The incidence of Diabetes mellitus has reached an alarming level despite having been a rare disease 50 years ago, attaining a global incidence of 151 million people per year (Piero et al, 2011). The number of people with diabetes is increasing due to population growth, aging, urbanization and increasing prevalence of obesity and physical inactivity (Wild et al, 2004). In 2004, an estimated 3.4 million people died from consequences of fasting high blood sugar. A similar number of deaths have been estimated for 2010 and more than 80% of diabetes deaths occur in low- and middle-income countries (WHO, 2011).
International Diabetes Federation (IDF) estimates that more than 366 million people have diabetes worldwide and this is expected to rise to 552 million by 2030. In sub-Saharan Africa where infectious disease has traditionally dominated health systems, rates of diabetes are soaring. The number of people living with diabetes in Africa has now risen to 14.7 million. This is expected to increase to 28 million by 2030, around a 90% increase. Worryingly it is people in their most productive years, under the age of 60, who are suffering the full force of the epidemic (IDF, 2011). In Kenya, study in 2009 showed a prevalence of 4.2% in the general population, with a prevalence of 2.2% of the rural areas and a prevalence of 12.2% in the urban population (Christensen et al, 2009).

The highest prevalence of diabetes in the Africa region is in the Island of Reunion (16.3 %) followed by the Seychelles. Some of Africa's most populous countries also have the highest number of people with diabetes, with Nigeria having the largest number at 3 million followed by South Africa at 1.9 million. Silver Bahendeka, Chair of IDF’s Africa region said “In Africa we need to encourage strengthening of our national health systems to encourage earlier diagnosis and prevent onset of later complications, which increase the social and economic burden on already poor areas” (IDF, 2011).

Urbanization with adoption of “western lifestyles” has been incriminated in the abandonment of the healthier “traditional lifestyles” by people in developing
countries. The traditional lifestyle was characterized by regular and vigorous physical activity accompanied by subsistence on high fiber, whole grain-based diet rich in vegetables and fruits. Urban or even “western lifestyles” in rural areas have resulted in overreliance on motorized transport and consumption of unhealthy diets rich in carbohydrates, fats, sugars and salts. These lifestyles have contributed to a rise in levels of obesity and overweight in the population increasing the risk for diabetes. For instance, the 2003 Kenya Demographic and Health Survey reported that about 20% of women and 7% of men in the country were overweight or obese (Maina et al, 2010).

Although diabetes is sometimes considered a condition of developed nations, the loss of life from premature death among persons with diabetes is greatest in developing countries. In developing countries it is people in the middle, productive years of their lives that are particularly affected by diabetes. In these countries three-quarters of all people with diabetes are under 65 years old and 25% of all adults with diabetes are younger than 44 years. In developed countries, more than half of all people with diabetes are older than 65 years, and only 8% of adults with diabetes are younger than 44 years (Beaglehole & Lefebvre, 2004).

Several pathogenic processes are involved in the development of diabetes. These range from autoimmune destruction of the β-cells of the pancreas with consequent insulin deficiency to abnormalities that result in resistance to insulin action. The
basis of the abnormalities in carbohydrate, fat, and protein metabolism in diabetes is deficient action of insulin on target tissues. Deficient insulin action results from inadequate insulin secretion and/or diminished tissue responses to insulin at one or more points in the complex pathways of hormone action. Impairment of insulin secretion and defects in insulin action frequently coexist in the same patient, and it is often unclear which abnormality, if either alone, is the primary cause of the hyperglycemia (ADA, 2012a).

The vast majority of cases of diabetes fall into two broad etiopathogenic categories. In one category, type I diabetes, the cause is an absolute deficiency of insulin secretion. Individuals at increased risk of developing this type of diabetes can often be identified by serological evidence of an autoimmune pathologic process occurring in the pancreatic islets and by genetic markers (ADA, 2012a).

Type I diabetes is considered a chronic immune-mediated disease with a subclinical prodrome of variable duration. It is characterized by selective loss of insulin-producing β-cells in the pancreatic islets in genetically susceptible subjects. The most important genes contributing to disease susceptibility are located in the human leukocyte antigen (HLA) class II locus on the short arm of chromosome 6. Nevertheless, only a relatively small proportion, that is, < 10%, of genetically susceptible individuals progress to clinical disease. This implies that additional factors are needed to trigger and drive β-cell destruction in genetically
predisposed subjects. However environmental factors have been implicated in the pathogenesis of type I diabetes both as triggers and potentiator of β-cell destruction, although the contribution of any individual exogenous factor has not yet been definitely proven (Knip et al, 2005).

In the other much more prevalent category, type II diabetes, the cause is a combination of resistance to insulin action and an inadequate compensatory insulin secretory response. In type II diabetes, a degree of hyperglycemia sufficient to cause pathologic and functional changes in various target tissues, but without clinical symptoms, may be present for a long period of time before diabetes is detected. During this asymptomatic period, it is possible to demonstrate an abnormality in carbohydrate metabolism by measurement of plasma glucose in the fasting state or after a challenge with an oral glucose load (ADA, 2012a).

At least 10 genetic loci have been linked to type II diabetes. In fact, individuals with an affected parent or siblings are at 3.5 time’s greater risk of developing diabetes than are those from diabetes free families. Until recently, type II diabetes occurred primarily in persons 40 years of age or older. However the appearance of type II diabetes has increased significantly among youths 20 years of age or younger reaching epidemic proportions. Coupled with prevalence of type II
diabetes among youths is the prevalence of obesity which also is increasing at epidemic levels (Dods, 2010).

Epidemiological studies show that type II diabetes is associated with overeating, especially when combined with obesity and under activity. Middle-aged people with diabetes eat significantly more and are fatter and less active than their non-diabetic siblings. The risk of developing type II diabetes increases tenfold in people with a body mass index (BMI) > 30kg/m². However, although the majority of patients with type II diabetes are obese, only a minority of obese people develop diabetes. Obesity probably acts as a diabetogenic factor (through increasing resistance to the action of insulin) only in those who are genetically predisposed both to insulin resistance and to β-cell failure (Frier & Fisher, 2010).

Other forms of diabetes include gestational diabetes (diabetes diagnosed during pregnancy) and other specific types of diabetes due to other causes, such as genetic defects in β-cell function, genetic defects in insulin action, diseases of the exocrine pancreas (such as cystic fibrosis), and drug- or chemical induced (such as in the treatment of HIV/ AIDS or after organ transplantation) (ADA, 2012b).

Symptoms of marked hyperglycemia include polyuria, polydipsia, weight loss, sometimes with polyphagia, and blurred vision. Impairment of growth and susceptibility to certain infections may also accompany chronic hyperglycemia
These symptoms are more marked in type I diabetes in which they develop rapidly in early stages while they are less marked in type II diabetes. As a result this type may be diagnosed several years after onset (WHO, 2011).

Acute, life-threatening consequences of uncontrolled diabetes are hyperglycemia with ketoacidosis or the non-ketotic hyperosmolar syndrome. Long-term complications of diabetes include retinopathy with potential loss of vision; nephropathy leading to renal failure; peripheral neuropathy with risk of foot ulcers, amputations, and Charcot joints; and autonomic neuropathy causing gastrointestinal, genitourinary, and cardiovascular symptoms and sexual dysfunction (ADA, 2012a).

Studies have shown that many complications of diabetes can be prevented or delayed through effective management (Beaglehole, & Lefebvre, 2004). The aim of the management of diabetes is to reduce the symptoms of diabetes and reduce blood glucose levels as uncontrolled hyperglycemia is associated with an increased risk of macrovascular and microvascular complications. Patients with type I diabetes always require treatment with exogenous insulin. For Type II diabetes, treatment options begin with diet modification and lifestyle interventions but often oral hypoglycemic agents or insulin or both are required as the disease progresses (Holden & Currie, 2012).
Five classes of oral agents are approved for the treatment of diabetes. Oral therapy is indicated in any patients in whom diet and exercise fail to achieve acceptable glycemic control. Although initial response may be good, oral hypoglycemic drugs may lose their effectiveness in a significant percentage of patients. The drug category includes sulfonylurea, biguanide, α-glucosidase inhibitor, thiazolidinedione and meglitinide. These drugs have various side effects such as sulfonylureas causes weight gain due to hyperinsulinemia, biguanide causes weakness, fatigue, lactic acidosis, α-glucosidase inhibitor may causes diarrhea while thiazolidinediones may increase LDL-cholesterol level. Insulin is usually added to an oral agent when glycemic control is suboptimal at maximal dose of oral medication. Weight gain and hypoglycemia are common side effect of insulin (Pandey et al, 2011).

Although insulin has been designated an essential drug by WHO, it is not yet universally accessible to all those who need it in the majority of countries of the world. Continuous access to insulin remains a major problem in many developing countries especially those in sub-Saharan Africa (Beaglehole & Lefebvre, 2004). Presently, there is growing interest in herbal remedies due to the side effects associated with the oral hypoglycemic agents (therapeutic agent) for the treatment of diabetes mellitus (Rao et al, 2010).
The World Health Organization (WHO) estimates that up to 80% of the world's population, mostly in developing countries, relies on traditional medicine practices for its health care needs. This is particularly true of the poorer sections of the population in developing countries because natural remedies are not only cheaper than modern medicines, but are often the only medicines available in remote rural regions (Musila, Kisangau & Muema, 2004).

In Kenya, traditional medicine continues to play a major role in Primary Health Care (PHC). More than 70% of the Kenyan population relies on traditional medicine as its primary source of health care, while more than 90% use medicinal plants at one time or another. It is more accessible than modern health facilities for most of the population in the country. It is relatively inexpensive, locally available, and usually accepted by the local communities as comparable to modern conventional medicine (Musila, Kisangau & Muema, 2004).

More than 400 traditional plant treatments for diabetes mellitus have been recorded, but only a small number of these have received scientific and medical evaluation to assess their efficacy (Bailey and Day, 1986). In diabetes, some herbal alternatives are proven to provide symptomatic relief and assist in the prevention of the secondary complication of the disease. Some herbs have also been proven to help in regeneration of β-cells and in overcoming resistance. In
addition to maintaining normal blood sugar level, some herbs are also reported to possess antioxidant activity and cholesterol lowering action (Pandey et al, 2011).

1.2 Problem statement and justification of the study

Diabetes mellitus is a chronic disorder with increasing rates of incidence and mortality. The disease remains a critical problem of public health today as it is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels. The conventional antidiabetic drugs are either expensive or unavailable or they have certain side effects which can lead to other complication to the patients. Many people in the developing countries have adopted using traditional and herbal medicines. These plants are cheap, locally available and assumed to have no or less toxic effect on the body.

However many plants have been traditionally in use without authentication of their anti-diabetes properties and assessment of their safety. As many conventional drugs have originated from medicinal herbs, studying these plants may lead to discovery of new drugs with higher efficacy and lower toxicity. In addition, many plants are being rendered extinct by human activity, which makes it necessary to study them before they become extinct and advocate for their conservation.
1.3 Research question
Do aqueous extracts of *Acacia nilotica*, *Aloe secundiflora*, *Zanha africana*, *Fuerstia africana* and *Kleinia squarrosa* exhibit hypoglycemic activity and are they safe?

1.4 Research hypothesis
Aqueous extracts of *Acacia nilotica*, *Aloe secundiflora*, *Zanha africana*, *Fuerstia africana* and *Kleinia squarrosa* exhibit hypoglycemic activity and can be safely used in management of diabetes.

1.5 Objectives
1.5.1 General objective
To determine the hypoglycemic activity and toxicity of aqueous extracts of *Acacia nilotica*, *Aloe secundiflora*, *Zanha africana*, *Fuerstia africana* and *Kleinia squarrosa* used in the management of diabetic mellitus by traditional health practitioners in Machakos County, Kenya.

1.5.2 Specific objectives
(i) To determine *in vivo* hypoglycemic activity of aqueous extracts of *Acacia nilotica*, *Aloe secundiflora*, *Zanha africana*, *Fuerstia africana* and *Kleinia squarrosa*. 
(ii) To evaluate the toxicity of aqueous extracts of *Acacia nilotica*, *Aloe secundiflora*, *Zantha africana*, *Fuerstia africana* and *Kleinia squarrosa*.

(iii) To determine the phytochemical components of aqueous extracts of *Acacia nilotica*, *Aloe secundiflora*, *Zantha africana*, *Fuerstia africana* and *Kleinia squarrosa*.

(iv) To determine the level of trace elements in the aqueous extracts of *Acacia nilotica*, *Aloe secundiflora*, *Zantha africana*, *Fuerstia africana* and *Kleinia squarrosa*. 
CHAPTER TWO

LITERATURE REVIEW

2.1 Classification of diabetes mellitus

The classification of diabetes includes four clinical classes: Type I diabetes (results from \( \beta \)-cell destruction, usually leading to absolute insulin deficiency), type II diabetes (results from a progressive insulin secretary defect on the background of insulin resistance), gestational diabetes mellitus (GDM) diagnosed during pregnancy, and other specific types of diabetes due to other causes such as genetic defects in \( \beta \)-cell function, genetic defects in insulin action, diseases of the exocrine pancreas (such as cystic fibrosis), and drug or chemical induced (such as in the treatment of AIDS or after organ transplantation) (ADA, 2012b); however assigning a type of diabetes to an individual often depends on the circumstances present at the time of diagnosis, and many diabetic individuals do not easily fit into a single class (ADA, 2012a).

2.1.1 Type I diabetes

Type I diabetes, previously known as insulin dependent diabetes or juvenile-onset diabetes accounts for only about 5-10% of all cases of diabetes; however, its incidence continues to increase worldwide and it has serious short-term and long-term implications. It is a condition in which pancreatic \( \beta \)-cell destruction usually leads to absolute insulin deficiency (Daneman, 2006; ADA, 2012a). The rate of \( \beta \)-cell destruction is quite variable, being rapid in some individuals (mainly infants
and children) and slow in others (mainly adults) (ADA, 2012a). The majorities of the patients are diagnosed and classified with type I diabetes within the first two decades of life, but an increasing number of cases are being recognized in older individuals (Piero et al, 2012a). Individuals with type I diabetes are especially prone to ketoacidosis, the excessive formation of keto-acids and low blood pH (acidosis) (Dods, 2010).

There are two forms of type I diabetes: type IA results from a cell-mediated autoimmune attack on β-cells, whereas type IB is far less frequent, has no known cause, and occurs mostly in individuals of Asian or African descent, who have varying degrees of insulin deficiency between sporadic episodes of ketoacidosis (Daneman, 2006). Some of these patients have permanent insulinopenia and are prone to ketoacidosis, but have no evidence of autoimmunity (ADA, 2012a). However, in both forms of type I diabetes hyperglycemia accompanied by the classical symptoms of diabetes occurs only when 70-90% of β-cells have been destroyed (Frier & Fisher, 2010).

The earliest sign of autoimmunity against β-cells, often detectable months or years before the appearance of clinical symptoms, is the presence of circulating antibodies against β-cell antigens. These antibodies are used as markers of diabetes risk (Pirot, Cardozo & Eizirik, 2008). The autoimmune nature of the disease process has invoked efforts aimed at arresting the disease process by
immune intervention strategies. Over the last quarter century much investigation has been directed at interdicting the type I diabetes disease process, both during the stage of evolution of the disease and at the time of disease onset (Piero et al, 2012a).

Genetic factors account for about one third of the susceptibility to type I diabetes. Over 20 different regions of human genome show some linkage with type I diabetes but most interest has focused on the human leukocyte antigen (HLA) region within the major histocompatibility complex (MHC) on the short arm of chromosome 6; this locus is designated as IDDM 1 (Frier & Fisher, 2010). The class II of MHC molecules are expressed at the surface of antigen presenting cells (APC) (that is, dendritic cells, macrophages and lymphocytes B) and are presented in the forms DR, DQ, and DP which are composed of two chains (α and β) encoded by genes A and B. The genetic predisposition to type I diabetes caused by HLA locus is related to specific polymorphism of DQ and DR forms of MHC class II molecules (Pirot, Cardozo & Eizirik, 2008).

HLA DR3 or DR4 or both types occur in 90% of patients with type I diabetes and susceptibility is greater when DR4 protein is produced in conjunction with a protein produced by the DQ locus, called DQw3.2; individuals who possess the DQw3.1 allele are less likely to acquire type I diabetes than are their DQw3.2 counterparts. Susceptibility to type I diabetes is increased further when DQ β-
chain lacks aspartic acid at position 57 and has arginine present at position 52 of the DQ α-chain (Dods, 2010). Some studies have also implicated other genes in type I diabetes such as CD25, PTPN22, IL2RA, which are involved in immune recognition of pancreatic islet antigens, T-cell development and immune regulation. The genes associated with type I diabetes overlap with those for other autoimmune disorders such as coeliac disease and thyroid disease consistent with clustering of these conditions in individuals or families (Frier & Fisher, 2010).

It was found that the pool of self reactive naive T-cells stays under the control of the immune system for several years and requires exposure to one or more environmental triggers that alter immune function, thereby initiating β-cell destruction (Daneman, 2006; Pirot, Cardozo & Eizirik, 2008). Knip et al (2005) reported that most initial auto-antibodies appear during the cold period in the fall and winter but rarely in the spring or in the summer which strongly points to the role of infectious agents with conspicuous seasonal variation as triggers of β-cell autoimmunity.

Accumulating evidence points to viral infections by enterovirus, rubella, mumps, rotavirus, parvovirus or cytomegalovirus as the most probable candidates for triggering β-cell autoimmunity and particular attention is given to the enteroviruses which have a specific tropism for the pancreas (Pirot, Cardozo & Eizirik, 2008).
Recently, a coxsackie B4 virus (an enterovirus) isolated from samples obtained from Type I diabetes patients was able to *in vitro* infect human islets from non-diabetic patients, causing impaired glucose stimulated insulin release (Dotta *et al.*, 2007). The mechanism by which enteroviruses may be involved in the pathogenesis of Type 1 diabetes is, however, elusive, but possible mechanisms include non-T-cell mediated β-cell destruction, induction of an enhanced auto-antigen specific T-cell response, molecular mimicry and bystander activation of auto-reactive T cells (Werf *et al.*, 2007).

Other environmental triggers include Bovine serum albumin (BSA), a major constituent of cow’s milk since children who are given cow’s milk early in infancy are more likely to develop type I diabetes than those who are breast fed. BSA may cross the neonatal gut and raise antibodies which cross-react with heat-shock protein expressed by β-cells (Frier & Fisher, 2010).

### 2.1.2 Type II diabetes

This form of diabetes, which accounts for 90-95% of those with diabetes, previously referred to as non-insulin-dependent diabetes, type II diabetes, or adult onset diabetes (ADA, 2012a). It is a more complex condition than type I diabetes because there is combination of resistance to action of insulin in liver and muscle together with impaired pancreatic β-cell function leading to relative insulin deficiency. Initially insulin resistance develops first and leads to elevated insulin
secretion in order to maintain normal blood glucose levels. In susceptible individuals the pancreatic β-cells are unable to sustain the increased demand for insulin and slowly progressive insulin deficiency develops (Frier & Fisher, 2010).

Until recently, type II diabetes occurred primarily in persons 40 years of age or older. However the appearance of type II diabetes has increased significantly among youths 20 years of age or younger reaching epidemic proportions. Coupled with prevalence of type II diabetes among youths is the prevalence of obesity which is also increasing at epidemic levels. Generally, unlike individuals with type I diabetes, those with type II diabetes are not dependent on insulin injections nor are they prone to ketoacidosis (Dods, 2010). This form of diabetes frequently goes undiagnosed for many years because the hyperglycemia develops gradually and at earlier stages it is often not severe enough for the patient to notice any of the classic symptoms of diabetes (ADA, 2012a).

The primary cause of insulin resistance remains unclear. However, intra-abdominal ‘central’ adipose tissue is metabolically active, and releases large quantities of free fatty acids (FFAs) which may induce insulin resistance because they compete with glucose as fuel supply for oxidation in peripheral tissues such as muscle. In addition adipose tissue releases a number of hormones (including a variety of peptides, called ‘adipokines’ because they are structurally similar to immunological cytokines) which act on specific receptors to influence sensitivity
to insulin in other tissues. Because visceral adipose tissue drains in the portal vein, central obesity may have a particular potent influence on insulin sensitivity in the liver, and thereby adversely affect gluconeogenesis and hepatic lipid metabolism (Frier & Fisher, 2010).

The other major type II diabetes risk factor is physical inactivity. Inactivity is associated with down-regulation of insulin-sensitive kinases and may promote accumulation of FFAs within skeletal muscle. Sedentary people are therefore more insulin-resistant than active people with the same degree of obesity. Moreover physical activity allows non-insulin dependent glucose uptake into the muscle, reducing the demand on the pancreatic β-cells to produce insulin (Frier & Fisher, 2010).

It has long been known that type II diabetes is, in part, inherited. Family studies have revealed that first degree relatives of individuals with type II diabetes are about 3 times more likely to develop the disease than individuals without a positive family history of the disease. It has also been shown that concordance rates for monozygotic twins, which have ranged from 60-90%, are significantly higher than those for dizygotic twins. Thus, it is clear that type II diabetes has a strong genetic component. Several susceptible genes have been identified which include: PPARγ (peroxisome proliferator-activated receptor-γ), ABCC8 (ATP binding cassette, subfamily C, member 8), KCNJ11 (the potassium channel,
Epidemiological studies show that type II diabetes is associated with overeating, especially when combined with obesity and under-activity. Middle-aged people with diabetes eat significantly more and are fatter and less active than their non-diabetic siblings. The risk of developing type II diabetes increases tenfold in people with a body mass index (BMI) > 30 kg/m². However, although the majority of patients with type II diabetes are obese, only a minority of obese people develop diabetes. Obesity probably acts as a diabetogenic factor (through increasing resistance to the action of insulin) only in those who are genetically predisposed both to insulin resistance and to β-cell failure (Frier & Fisher, 2010).

2.1.3 Gestational diabetes

Gestational diabetes mellitus (GDM) is defined as any degree of glucose intolerance with onset or first recognition during pregnancy. The definition does not exclude the possibility that unrecognized glucose intolerance may have antedated or begun concomitantly with the pregnancy. Approximately 7% of all pregnancies are complicated by GDM, resulting in more than 200,000 cases annually. The prevalence may range from 1 to 14% of all pregnancies, depending on the population studied and the diagnostic tests employed (ADA, 2012a).
Gestational diabetes is driven from a women’s inability to secrete sufficient insulin to compensate for the increased nutritional needs of pregnancy, the greater numbers of adipose cells during pregnancy, and the pregnancy associated secretion of increased quantities of hyperglycemic hormones, including human placental lactogen, cortisol, prolactin and progesterone. This results in a nearly fourfold increase in the need for insulin secretion. When this need for the additional insulin is not totally met, hyperglycemia develops in a pregnant woman (Dods, 2010). Shortly after delivery, glucose homoeostasis is restored to non-pregnancy levels, but affected women remain at high risk of developing type II diabetes mellitus in the future (Bellamy et al, 2009).

In addition repeated pregnancy increases the likelihood of developing irreversible diabetes, particularly in obese women. A clear relationship exists between maternal blood glucose and prenatal morbidity for the baby. Maternal glucose crosses the placenta and is an important fuel for the developing fetus. Elevated maternal blood glucose promotes fetal insulin production and hence stimulates fetal growth (macrosomia), which may complicate labour and delivery, resulting in higher caesarean section rate. Fetal hyperinsulinemia may also result in neonatal hypoglycemia (Frier & Fisher, 2010). The greatest risk for intrauterine death or neonatal mortality occurs when the mother’s glucose levels are greater than 110 mg/dL when fasting or greater than 120 mg/dL after a meal. A child who
has been exposed to hyperglycemia in the uterus is at greater risk for the development of diabetes later in life (Dods, 2010).

2.1.4 Other specific types of diabetes

This category encompasses diabetes mellitus that is caused by other causes such as: genetic defect of β-cell function, genetic defect in insulin action, pancreatic diseases (such as pancreatitis, neoplastic disease and cystic fibrosis), excessive production of hormonal antagonists to insulin (such as growth hormone, glucocorticoids, glucagon, catecholamine, and thyroid hormones), drug induced and virus infections (Frier & Fisher, 2010).

2.2 Symptoms of diabetes mellitus

The disease has several symptoms which may occur suddenly. These include excessive excretion of urine (polyuria), thirst (polydipsia), constant hunger (polyphagia), weight loss, vision changes and fatigue. These symptoms are more marked in type I diabetes in which they develop rapidly in early stages while they are less marked in type II diabetes. As a result this type may be diagnosed several years after onset (WHO, 2011).

Thirst develops because of osmotic effects; sufficiently high glucose (above the 'renal threshold') in the blood is excreted by the kidneys but this requires water to carry it and causes increased fluid loss, which must be replaced. The lost blood
volume will be replaced from water held inside body cells, causing dehydration (Anon, 2005). Uncontrolled diabetes is associated with an increased susceptibility to infections and patients may present with skin sepsis (boils) or genital candidiasis, and complain of pruritus vulvae or balanitis (Frier & Fisher, 2010).

Especially dangerous symptoms in diabetics include the smell of acetone on the patient's breath (a sign of ketoacidosis), Kussmaul breathing (a rapid, deep breathing), and any altered state of consciousness or arousal (hostility and mania are both possible, as is confusion and lethargy). The most dangerous form of altered consciousness is the so-called "diabetic coma" which produces unconsciousness. Early symptoms of impending diabetic coma include polyuria, nausea, vomiting and abdominal pain, with lethargy and somnolence a later development, progressing to unconsciousness and death if untreated (Anon, 2005).

2.3 Diagnosis of diabetes mellitus

2.3.1 Blood tests

Various blood test methods are routinely used in diagnosis of diabetes mellitus. These include:

2.3.1.1 Fasting plasma glucose

Fasting glucose is directly proportional to the severity of diabetes mellitus. During this test, blood is drawn from a vein in the patient's arm after the patient has not
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eaten for at least eight hours, usually in the morning before breakfast. The red blood cells are separated from the sample and the amount of glucose is measured in the remaining plasma. The fasting glucose test is usually repeated on another day to confirm the results (Piero et al, 2012b). Repeated fasting plasma glucose greater than 126 mg/dL are strongly suggestive of diabetes and values from 100 to 126 mg/dL are suggestive of impaired fasting glucose (Dods, 2010).

2.3.1.2 Postprandial plasma glucose test

Diabetes is detected more readily when carbohydrate metabolic capacity is tested. This can be done by stressing the system with defined glucose load. Measurement of the rate at which the glucose load is cleared from the blood, as compared with rate of glucose clearance in healthy persons, detects impairment in glucose metabolism. A meal high in carbohydrates is often used as the carbohydrate load, although a 75g glucose drink is usually preferred over a meal. Two consecutive postprandial tests are recommended for diagnosis (Dods, 2010).

Blood is drawn at 2 hours after ingestion of the meal or glucose drink. Two postprandial tests with glucose levels of 200 mg/dL or higher at two hours are suggestive of diabetes. Postprandial glucose test, although widely used for detection of diabetes, is highly inaccurate because of several variables that are difficult to control or adjust for. These variables include age, weight, previous diet, activity, illness, medications, time of day that the test is conducted and
actual size of the glucose dose. When a meal is used as the load the effective glucose load depend on digestion of the disaccharides and polysaccharides and their subsequent absorption from the intestinal tract (Dods, 2010).

2.3.1.3 Oral glucose tolerance test

The oral glucose tolerance test (OGTT) evaluates glucose clearance from the circulation after glucose loading under defined and controlled conditions. The committee on statistics of the American Diabetes Association has standardized the test. Standard conditions call for minimum carbohydrate intake of 150 g/day for 3 days before the test (Dods, 2010). The patient should have been fasting for the previous 8-14 hours. A zero time (baseline) blood sample is drawn. The patient is given a glucose solution, which is drunk within 5 minutes. Blood is drawn at intervals for measurement of glucose (blood sugar), and sometimes insulin levels (Piero et al, 2012b).

The glucose load should consist of glucose only. Some commercial preparations labeled “100 grams glucose equivalent” contains disaccharides and polysaccharides. The rate at which these saccharides are hydrolyzed and absorbed from intestinal tract varies from person to person. Such a preparation is not desirable for individuals with pancreatic or malabsorptive disorders (Dods, 2010). The intervals and number of samples vary according to the purpose of the test. For simple diabetes screening, the most important sample is the 2-hour sample.
The zero and 2-hour samples may be the only ones collected. In a non-diabetic, the level of glucose in the blood goes up immediately after the drink and then decreases gradually as insulin is used by the body to metabolize or absorb the sugar. In a diabetic, the glucose in the blood goes up and stays high after drinking the sweetened liquid (Piero et al, 2012b). If the 2-hour blood glucose level is between 140 and 199 mg/dL, the person has a type of pre-diabetes called impaired glucose tolerance (IGT). If confirmed by a second test, a 2-hour glucose level of 200 mg/dL or above means a person has diabetes (National Diabetes Information Clearinghouse “NDIC”, 2014).

During the test, the patient must be ambulatory, since inactivity decreases glucose tolerance. The test can also be affected by illness, abnormalities of such hormones as thyroxine, growth hormone, cortisol, and catecholamines, drugs and medications such as oral contraceptives, salicylates, nicotinic acid, diuretics and hypoglycemic agents and testing time (Piero et al, 2012b). The best time to conduct the test is between 7 AM and noon. Evaluation criteria should be adjusted for age, if adjustments for age are not made, about 80% of persons over 60 years of age will be judged diabetic (Dods, 2010).

2.3.1.4 Intravenous glucose tolerance test
The intravenous glucose tolerance test is used for persons with malabsorptive disorders or previous gastric or intestinal surgery. Glucose is administered
intravenously over 30 minutes, using a 20% solution. A glucose load of 0.5g/kg of body weight is used. Non-diabetics respond with a plasma glucose level of 200 to 250 mg/dL. Discontinuation of the glucose loading leads to a decrease in plasma levels with fasting levels reached at about 90 minutes. Diabetics demonstrate plasma glucose level above 250 mg/dL during administration of the load. On discontinuation of the loading, plasma glucose levels of diabetics also return to fasting levels at about 90 minutes. An alternative procedure called the Soskin method uses 50% glucose delivered intravenously within 3 to 5 minutes. The glucose load used is 0.3 g/kg of body weight. Non-diabetics re-establish fasting levels in less than 60 minutes after discontinuing the glucose infusion. In diabetics fasting levels are established significantly later than 60 minutes (Dods, 2010).

2.3.1.5 O’Sullivan-mahan glucose challenge test

O’Sullivan-Mahan glucose challenge test is used frequently to detect gestational diabetes. A 50g load of glucose is given to a fasting patient and a blood glucose measurement is made 1 hour after dosage. A plasma glucose level above 140 mg/dL suggests gestational diabetes, and a full oral glucose tolerance test is recommended for such patients (Dods, 2010).

2.3.1.6 Glycated hemoglobin

A minor hemoglobin derivative called HbA1c is produced by glycation, the covalent binding of glucose to hemoglobin. Because this reaction is non-
enzymatic and because the red cell is completely permeable to glucose, the quantity of HbA1c formed is directly proportional to the average plasma glucose concentration that the red blood cell is exposed to during its 120-day life span. Thus, in long term hyperglycemia, HbA1c constitutes a higher percentage of total hemoglobin than in normoglycemia and transient elevations in plasma glucose only mildly affect HbA1c levels (Dods, 2010).

Glycated hemoglobin provides an accurate and objective measure of glycemic control over period of weeks to months. This allows assessment of glycemic control by repeated measurements every few months in patients with known diabetes (Frier & Fisher, 2010). The HbA1C test reflects the average of a person’s blood glucose levels over the past 3 months and does not show daily fluctuations. The HbA1C test is more convenient for patients than the traditional glucose tests because it does not require fasting and can be performed at any time of the day (NDIC, 2014).

The HbA1c test result is reported as a percentage. A rise of 1% in HbA1c corresponds to an approximate average increase of 2 mM (36 mg/dL) in blood glucose. The higher the percentage, the higher a person’s blood glucose levels have been. A normal HbA1C level is below 5.7 percent. HbA1C of 5.7 to 6.4 percent indicates pre-diabetes. People diagnosed with pre-diabetes may be retested in 1 year. People with HbA1C below 5.7 percent may still be at risk for
diabetes, depending on the presence of other characteristics that put them at risk, also known as risk factors. People with HbA1C above 6.0 percent should be considered at very high risk of developing diabetes. A level of 6.5 percent or above means a person has diabetes (Frier & Fisher, 2010; NDIC, 2014).

The HbA1C test can be unreliable for diagnosing or monitoring diabetes in people with certain conditions known to interfere with the results. Interference should be suspected when HbA1C results seem very different from the results of a blood glucose test. People of African, Mediterranean, or Southeast Asian descent or people with family members with sickle cell anemia or a thalassemia are particularly at risk of interference. False HbA1C test results may also occur in people with other problems that affect their blood or hemoglobin such as chronic kidney disease, liver disease, or anemia (NDIC, 2014).

2.3.1.7 Plasma insulin test

Fasting plasma insulin levels in type I diabetics are usually low. Those of type II diabetics are low only when fasting plasma glucose levels exceed 250mg/dL. Otherwise, they are normal or even elevated. A glucose challenge separates type I diabetics from type II diabetics. Glucose loading elicits no significant insulin response for type I diabetics and a delayed, exaggerated response in type II diabetics (Dods, 2010).
Close monitoring of blood glucose levels has been aided by the development of increasingly accurate and reliable bedside glucose monitors, for use in hospital or by the patient. Correct use of such device should minimize the wide variations in blood glucose experienced by persons with diabetes and as a result hyperglycemia events and even long term complications of diabetes (Dods, 2010). A small needle or lancet is used to prick the finger and a drop of blood is collected and analyzed by a monitoring device. Some patients may test their blood glucose levels several times during a day and use this information to adjust their diet or doses of insulin (Piero et al, 2012b).

In a consensus statement on blood glucose monitoring, many insulin-treated populations have been recommended for self-monitoring programs. These include pregnant women, patients with unstable diabetes, patients with histories of severe ketosis or hypoglycemia especially those who do not demonstrate warning symptoms of hypoglycemia, patients receiving intensive insulin therapy and patients with abnormal renal thresholds for glucose (Dods, 2010; Piero et al, 2012b).

2.3.2 Urine tests

Urine tests are undertaken to analyze glucose, ketone bodies, and proteins in the urine (Piero et al, 2012b). Testing urine for glucose with dipsticks is a common screening procedure for detecting diabetes. If possible, testing should be
performed on urine passed 1-2 hours after a meal to maximize sensitivity (Frier & Fisher, 2010). However urinary glucose is a poor marker for Diabetes. The normal renal threshold for glucose is 180 mg/dL. Blood glucose levels must exceed this value before excessive glucose is apparent in the urine. Further complicating this picture is the fact that the renal threshold in persons with diabetes often is increased to above 300 mg/dL (Dods, 2010).

Ketone bodies can be identified by the nitroprusside reaction, which measures acetoacetate, using either tablets or dipsticks. Ketonuria may be found in normal people who have been fasting or exercising strenuously for long periods, who have been vomiting repeatedly, or who have been eating a diet high in fat and low in carbohydrate. Ketonuria is therefore not pathognomonic of diabetes but, if associated with glucosuria, the diagnosis of diabetes is highly likely. In diabetic ketoacidosis ketones can also be detected in plasma using dipsticks (Frier & Fisher, 2010).

Standard dipsticks testing for albumin detects urinary albumin at concentrations > 300 mg/L, but smaller amounts (microalbuminuria, can only be measured using specific albumin dipsticks or by quantitative biochemical laboratory measurement. Microalbuminuria or Proteinuria, in the absence of urinary tract infection, is an important indicator of the development of diabetic nephropathy and or increased risk of macrovascular disease (Frier & Fisher, 2010).
2.4 Complications of diabetes

Diabetes mellitus is a very expensive disease and has profound implications in terms of long-term microvascular and macrovascular complications and their associated cost. These complications reduce both life expectancy and quality of life (Piero et al., 2012c). The following are the most common complications of diabetes mellitus.

2.4.1 Diabetes retinopathies

Diabetic retinopathy is retinopathy (damage to the retina) caused by complications of diabetes mellitus, which can eventually lead to blindness. It is an ocular manifestation of systemic disease which affects up to 80% of all patients who have had diabetes for 10 years or more (Joy, Kumar & Rajasekhar, 2011). Diabetes retinopathy is the most frequent cause of new cases of blindness among adults aged 20-74 years. During the first two decades of the disease, nearly all patients with type I diabetes and more than 60% of patients with type II diabetes have retinopathy (Fong et al, 2004).

Hyperglycemia increases retinal blood flow and metabolism and has direct effect on retinal endothelial cells and pericyte loss, which impairs vascular auto-regulation. The resulting uncontrolled blood flow initially dilates capillaries but also increases production of vasoactive substances and endothelial cell proliferation, resulting in capillary closure. This cause chronic retinal hypoxia and
stimulates production of growth factors, including vascular endothelial growth factor (VEGF), which plays a major role in stimulating the deleterious changes of endothelial cell growth (causing new vessel formation), and increased vascular permeability (causing retinal leakage and exudation) (Frier & Fisher, 2010). The development of the disease occurs in stages which are:

(i) Non-proliferative diabetic retinopathy (NPDR):
Non-proliferative diabetic retinopathy is the earliest stage of diabetic retinopathy. Damaged blood vessels fluid from blood plasma and small amounts of red blood cells will drain into the retina. Cholesterol, triglycerides, and proteins from blood may leak into the retina forming hard exudates (Joy, Kumar & Rajasekhar, 2011). In this stage central vision is affected by any of the following: Cholesterol and protein deposits in the central retina (macula), micro-aneurysms (small bulges in blood vessels of the retina may leak), retinal hemorrhages (tiny spots of blood that may form in the central macula), macular edema (swelling/thickening of macula), and macular ischemia (closing of small blood vessels or capillaries) (Joy, Kumar & Rajasekhar, 2011). As many more blood vessels are blocked, in response affected areas of retina send signals for growth of new vessels (National Eye Institute “NEI”, 2009).
(ii) Proliferative diabetic retinopathy (PDR)

Proliferative diabetic retinopathy is the later stage of diabetic retinopathy. Growth of new blood vessels occur, however these blood vessels are abnormal and fragile and cause blood leakage which leads to severe vision loss and even blindness. As these vessels grow a scar-like tissue (fibro-vascular proliferation) develops which can lead to retinal detachment (NEI, 2009; Joy, Kumar & Rajasekhar, 2011).

2.4.2 Diabetes neuropathy

Diabetic neuropathies are a family of nerve disorders caused by diabetes. People with diabetes can, over time, develop nerve damage throughout the body. Some people with nerve damage have no symptoms. Others may have symptoms such as pain, tingling, or numbness-loss of feeling-in the hands, arms, feet, and legs. Nerve problems can occur in every organ system, including the digestive tract, heart, and sex organs (NDIC, 2009). Although there is some evidence that the central nervous system is affected in long-term diabetes, the clinical impact of diabetes is mainly manifest in the peripheral nervous system (Frier & Fisher, 2010).

Diabetic neuropathy results from decreased motor and sensory nerve conduction velocities caused by axonal degeneration and demyelination. Of the many types of diabetic neuropathy, both peripheral and autonomic, distal symmetric sensorimotor polyneuropathy is the most frequent. Besides causing pain in its
early stages, this type of neuropathy eventually results in the loss of peripheral sensation. The combination of decreased sensation and peripheral arterial insufficiency often leads to foot ulceration and eventual amputation (Piero et al, 2012c). Autonomic neuropathy is a more serious condition and has several clinical manifestations which include: resting tachycardia, exercise intolerance, orthostatic hypotension, constipation, gastroparesis, erectile dysfunction, sudomotor dysfunction and impaired neurovascular function (ADA, 2012b).

2.4.3 Angiopathy

Refers to damage to lining (basement membrane) of blood vessels. This damage increases the risk of coronary heart diseases and can lead to nephropathy and retinopathy (Dods, 2010). There are two types of angiopathies: macroangiopathy and microangiopathy. In macro-angiopathy, fat and blood clots build up in the large blood vessels, stick to the vessel walls, and block the flow of blood. In microangiopathy, the walls of the smaller blood vessels become so thick and weak that they bleed, leak protein, and slow the flow of blood through the body. Then the cells, for example, the ones in the center of the eye, do not get enough blood and are damaged (Piero et al, 2012c).

2.4.4 Diabetes nephropathy

Nephropathy refers to damage to the capillaries associated with glomerulus (filtering apparatus of the nephron); this damage is associated with a reduction in
the filtering capability of the kidneys. Capillary damage is caused by angiopathy (Dods, 2010). Persistence albuminuria in the range of 30-299 mg/day (microalbuminuria) has been shown to be the earliest stage of diabetes nephropathy in type I diabetes and a marker of the development of nephropathy in type II diabetes. Patients with micro-albuminuria which progresses to macroalbuminuria (≥ 300mg/day) are likely to progress to the end-stage renal disease (ADA, 2012b).

Initially, there is renal hypertrophy, with expansion of the glomeruli, including the mesangium and glomerular basement membrane, and an increase in kidney size. Glomerular composition changes more slowly, leading to characteristic mesangial expansion, thickening of the glomerular basement membrane, and afferent and efferent arteriosclerosis. With more advanced nephropathy (progressive proteinuria), glomerular closure occurs (Nathan, 1993). All patients with diabetes have the potential for renal impairment unless proven otherwise. Since chronic blood pressure elevation contributes to the decline in renal function, referral of patients with diabetes who are hypertensive for long-term blood pressure management is extremely important (Piero et al, 2012c).

2.4.5 Diabetic Ketoacidosis

Diabetic ketoacidosis (DKA) is an acute, major, life-threatening complication of diabetes that mainly occurs in patients with type I diabetes, but it is not
uncommon in some patients with type II diabetes. This condition is a complex disordered metabolic state characterized by hyperglycemia, ketoacidosis, and ketonuria. DKA usually occurs as a consequence of absolute or relative insulin deficiency that is accompanied by an increase in counter-regulatory hormones (that is glucagon, cortisol, growth hormone, and epinephrine). This type of hormonal imbalance enhances hepatic gluconeogenesis, glycogenolysis, and lipolysis. Hepatic gluconeogenesis, glycogenolysis secondary to insulin deficiency, and counter-regulatory hormone excess result in severe hyperglycemia; while lipolysis increases serum free fatty acids (Osama, 2015).

Fatty acid degradation increases as it becomes the major source of energy for the cell. Increased fatty acid catabolism produces excessive acetyl-CoA, which is metabolized to produce abnormal levels of ketoacids (ketosis) (Dods, 2010). Ketones include acetone, β-hydroxybutyrate, and acetoacetate. Progressive rise of blood concentration of these acidic organic substances initially leads to a state of ketonemia, although extracellular and intracellular body buffers can limit ketonemia in its early stages, as reflected by a normal arterial pH associated with a base deficit and a mild anion gap. When the accumulated ketones exceed the body's capacity to extract them, they overflow into urine (that is ketonuria). If the situation is not treated promptly, a greater accumulation of organic acids leads to frank clinical metabolic acidosis (that is ketoacidosis), with a drop in pH and bicarbonate serum levels (Osama, 2015).
Ketones, in particular, \( \beta \)-hydroxybutyrate induce nausea and vomiting that consequently aggravate fluid and electrolyte loss already existing in DKA. Moreover, acetone produces the fruity breath odor that is characteristic of ketotic patients. Hyperglycemia, osmotic diuresis, serum hyperosmolarity, and metabolic acidosis result in severe electrolyte disturbances. The most characteristic disturbance is total body potassium loss. Potassium loss is caused by a shift of potassium from the intracellular to the extracellular space in an exchange with hydrogen ions that accumulate extracellularly in acidosis. Much of the shifted extracellular potassium is lost in urine because of osmotic diuresis. Patients with initial hypokalemia are considered to have severe and serious total body potassium depletion. High serum osmolarity also drives water from intracellular to extracellular space, causing dilutional hyponatremia. Sodium also is lost in the urine during the osmotic diuresis (Osama, 2013).

2.4.6 Hyperlipidemia and atherosclerosis

Abnormalities in circulating lipids are seen in diabetics. The most characteristic pattern is that of increased very low density lipoprotein (VLDL), which is manifested by elevation of plasma triacylglycerols and cholesterol but the former predominates. Insulin deficiency leads to hyperglycemia and enhanced lipolysis. Glucose and free fatty acids flow to the liver, where they are utilized for VLDL synthesis (Piero et al, 2012c). High-density lipoprotein (HDL) levels have been reported to be significantly lower in diabetic than in nondiabetic persons. These
results are consistent with the high incidence and natural history of atherosclerotic coronary heart diseases in persons with diabetes, and with higher death rates from myocardial infarction in diabetes individuals (Dods, 2010).

Atherosclerosis is a condition caused by cholesterol build up and inflammation in arteries. This leads to lesions in arteries called plaques. These plaques are made up of excess cholesterol, other fats, and inflammatory cells in the artery wall. These plaques can lead to narrowing of arteries and cause symptoms from decreased blood flow such as chest pain (angina) or pain in legs (claudication). In addition these plaques can sometimes suddenly become unstable and rupture, leading to heart attacks and strokes. Atherosclerotic occlusive vascular disease is the most common complication of diabetes especially in type II diabetic patients. The lesions of atherosclerosis 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 (Piero et al, 2012c).

2.4.7 Diabetic foot ulcers

Diabetic foot ulcers contribute significantly to the morbidity and mortality of patients with diabetes mellitus (Piero et al, 2012c). Foot ulceration occurs as a result of trauma (often trivial) in the presence of neuropathy and/or peripheral vascular disease, with infection occurring as a secondary phenomenon following disruption of the protective epidermis. Most ulcers develop at the site of a plaque
of callus skin beneath which tissue necrosis occur and eventually breaks through the surface. In most cases multiple components are involved, but sometimes neuropathy or ischemia predominates. Ischemia alone accounts for a minority of foot ulcers in diabetic patients, with most being either neuropathic or neuro-ischemic in type (Frier & Fisher, 2010).

2.5 Management of diabetes

The aim of the management of diabetes is to reduce the symptoms of diabetes and reduce blood glucose levels as uncontrolled hyperglycemia is associated with an increased risk of macrovascular and microvascular complications. Patients with type I diabetes always require treatment with exogenous insulin. For type II diabetes, treatment options begin with diet modification and lifestyle interventions but often oral hypoglycemic agents or insulin or both are required as the disease progresses (Holden & Currie, 2012).

2.5.1 Insulin therapy

Insulin was discovered in 1921 and transformed the management of type I diabetes. Until the 1980s insulin was obtained by extraction and purification from pancreata of cows and pigs (bovine and porcine insulin respectively) and some patients still prefer animal insulin. Recombinant DNA technology enabled large-scale production of human insulin. More recently, the amino acid sequence of
insulin has been altered to produce analogues of insulin, which differ in their rate of absorption from the site of injection (Frier & Fisher, 2010).

Depending on the rate of absorption, peak time and duration of action insulin is classified into four types: 1) Rapid-acting insulin, begins to work about 15 minutes after injection, peaks in about 1 hour, and continues to work for 2 to 4 hours. 2) Regular or Short-acting insulin usually reaches the bloodstream within 30 minutes after injection, peaks anywhere from 2 to 3 hours after injection, and is effective for approximately 3 to 6 hours. 3) Intermediate-acting insulin generally reaches the bloodstream about 2 to 4 hours after injection, peaks 4 to 12 hours later, and is effective for about 12 to 18 hours. 4) Long-acting insulin reaches the bloodstream several hours after injection and tends to lower glucose levels fairly evenly over a 24-hour period (ADA, 2012c).

In most patients, insulin is injected subcutaneously several times a day into the anterior abdominal wall, upper arms, outer thighs and buttocks. Accidental intramuscular injection often occurs in children and thin adults. The rate of absorption of insulin may be influenced by many factors other than the insulin formulation, including the site, depth and volume of injection, skin temperature (warming), local massage and exercise. Absorption is delayed from areas of lipohypertrophy at injection sites, which results from the local trophic action of insulin, so repeated injection at the same site should be avoided. Other routes of
administration (intravenous and intraperitoneal) are reserved for specific circumstances (Frier & Fisher, 2010).

Inhaled insulin was introduced in 2006 as an alternative to traditional insulin injection and continuous subcutaneous insulin infusion. This preparation consists of human insulin inhalation powder, which is administered using an inhaler. It has an onset of action similar to rapid-acting insulin analogs with duration of glucose-lowering activity comparable to subcutaneously administered regular human insulin. Inhaled insulin can be used in combination with long-acting analogs to treat hyperglycemia in patients with type I diabetes mellitus and can be used as monotherapy or in combination with oral agents and long-acting insulin analogs to treat patients with type II diabetes mellitus. Inhaled insulin is contraindicated in patients who have smoked within the previous 6 months or who have unstable or poorly controlled pulmonary disease (American Association of Clinical Endocrinology “AACE”, 2007).

Patients with type I diabetes require lifelong treatment with insulin to promote glucose utilization. Optimal diabetic control requires frequent self-monitoring of blood glucose. Frequent monitoring allows for rational adjustments in insulin doses. A multiple injection regimen, in which regular or lispro insulin is adjusted before each meal and intermediate-acting insulin given at bedtime, is used to provide more flexibility and achieve better glycemic control. With a multiple
injection regimen, patients add or subtract regular or lispro insulin (called compensatory doses) from their basic insulin dose in response to the immediate blood glucose level before the meal (Piero et al, 2012d).

Insulin is removed mainly by the liver and also the kidneys; plasma insulin concentrations are elevated in patients with liver disease or renal failure. The rate of clearance is also affected by binding to insulin antibodies (associated with the use of animal insulin). The complications of insulin therapy include: hypoglycemia, weight gain, peripheral edema (insulin treatment causes salt and water retention in the short term), insulin antibodies (animal insulins), local allergy (rare) and lipodystrophy at injection sites (Frier & Fisher, 2010).

2.5.2 Oral hypoglycemic drugs

2.5.2.1 Biguanides

Metformin is the only biguanide available. It is now used as first line for type II diabetes, irrespective of body weight. Metformin is also used increasingly as an adjunct to insulin therapy in obese patients with type I diabetes. The mechanism of action of metformin has not been precisely defined. It has no hypoglycemic effect in nondiabetic individuals, but in diabetes, insulin sensitivity and peripheral glucose uptake are increased, possibly through inhibition of mitochondrial respiration and activation of AMP-regulated kinase (AMPK) in muscle. There is
some evidence that it also impairs glucose absorption by the gut and inhibits hepatic gluconeogenesis (Frier & Fisher, 2010).

Metformin originally derived from a medicinal plant *Galega officinalis* (Piero *et al.*, 2012d). It has been shown to lower HbA1c levels by 1% to 2% and also confers other non-glycemic benefits such as decreasing low-density lipoprotein cholesterol (LDL-C) levels, triglyceride levels (AACE, 2007). Administration of metformin is not associated with a rise in body weight and it may be beneficial for the overweight or obese patients. In addition, as the glucose lowering effect of metformin is synergistic with that of sulphonylureas, they can be combined when either alone has proved in adequate. It can also be given with most other anti-diabetic medications. Metformin is given with food, usually starting with 500 mg 12 hourly, gradually increased as required to maximum of 1 g 8-hourly (Frier & Fisher, 2010).

Adverse effects of metformin include gastrointestinal distress such as abdominal pain, nausea, and diarrhea. These effects occur in up to 50% of patients; however, their frequency can be minimized with slow titration of therapy and food consumption. Metformin should not be used in patients who are at increased risk for lactic acidosis because of renal impairment. Metformin use should also be avoided in patients with hepatic dysfunction, congestive heart failure, metabolic acidosis, dehydration, and alcoholism. In addition, metformin should be
temporarily withheld in patients with acute illness or those undergoing radiocontrast studies or surgery (AACE, 2012).

2.5.2.2 Sulphonylureas

These agents reduce blood glucose by increasing insulin secretion from pancreatic β-cells in patients with residual β-cell function. All are well absorbed and their half-life and duration of action vary by agent (Piero et al, 2012d). Sulphonylureas bind to sulphonylurea receptors on the surface of pancreatic β-cells, and cause the voltage-dependent potassium adenosine triphosphate channels to close, which facilitates cell-membrane depolarization, calcium entry into the cell, and insulin secretion. Sulfonylurea therapy reduces HbA1c levels by 1% to 2% (AACE, 2007).

The main difference between the individual compounds lies in their potency, duration of action and cost. Tolbutamide, the mildest of the first generation sulphonylureas, is very well tolerated. Its duration of action is relatively short, it is usually administered 8 or 12-hourly, and it is a useful in the elderly in whom the risk and the consequences of inducing hypoglycemia is greater. Chlorpropamide has a biological half-life of about 36 hours and is taken once daily, but may cause severe and prolonged hypoglycemia and is rarely used. Of the second generation sulphonylureas, gliclazide and glipizide cause few side effects, but glibenclamide is prone to induce severe hypoglycemia and should be avoided in elderly. Newer
long acting preparations such as glimepiride and modified-release of gliclazide can be administered once daily with no apparent increased risk of hypoglycemia (Frier & Fisher, 2010).

Sulphonylureas are approved for use as monotherapy and in combination with most other oral drug classes and insulin; they are not approved for use in combination with glinides. Because most sulphonylurea agents are metabolized by the liver and cleared by the kidney, they should be used cautiously in patients with hepatic or renal impairment (AACE, 2007). The side effects of sulphonylureas include hypothyroidism and thyromegaly (through inhibition of organic iodine binding), cardiovascular disorders, skin infections, hematological disorders; cholestatic jaundice, hyponatremia, and water retention. Besides, sulphonylurea therapy is associated with weight gain due to hyperinsulinemia, which has been implicated as a cause of secondary drug failure (Piero et al, 2012d).

2.5.2.3 Glinides

Glinides employ a mechanism of action similar to sulphonylureas to facilitate glycemic control; however, they have a much shorter metabolic half-life. Glinides stimulate a rapid but short-lived release of insulin from pancreatic β-cells that lasts 1 to 2 hours. When taken at meals, these agents attenuate postprandial glucose excursions and decrease the risk of hypoglycemia during the late
postprandial phase because less insulin is secreted several hours after the meal. Therefore, use of glinides should target postprandial blood glucose levels rather than fasting blood glucose levels (AACE, 2007).

Two glinides are commercially available: nateglinide and repaglinide. The efficacy of repaglinide is similar to that of sulphonylureas, and is a suitable option for patients with severe sulfa allergy who are not candidates for sulphonylurea therapy. Nateglinide appears to be somewhat less potent. Glinides are used as monotherapy or in combination with metformin. They are metabolized by the liver and cleared by the kidney and should be used with caution in patients with hepatic or renal impairment. However, repaglinide is only minimally cleared by the kidney and can, therefore, be used safely in patients with even severe renal impairment (AACE, 2007; Piero et al, 2012d).

2.5.2.4 α-Glucosidase inhibitors

α-Glucosidase inhibitors provide postprandial glucose control by decreasing the absorption of carbohydrates from the gastrointestinal tract. These agents work by inhibiting α-glucosidase, an enzyme located in the proximal small-intestinal epithelium that breaks down disaccharides and more complex carbohydrates. Through competitive inhibition of this enzyme, α-glucosidase inhibitors delay intestinal carbohydrate absorption, thus attenuating postprandial glucose excursions (AACE, 2007). Undigested sugar is delivered to the colon, where it is

α-Glucosidase inhibitor therapy reduces HbA1c levels by approximately 0.5% to 1.0% (AACE, 2007). These agents do not increase insulin levels or inhibit lactase. They do not cause weight gain and restore ovulation in women with an ovulation due to insulin resistance (Piero et al, 2012d). Acarbose and miglitol are available and are taken with each meal. Both lower postprandial blood glucose and modestly improve overall glycemic control. They can be combined with sulphonylurea (Frier & Fisher, 2010).

Acarbose and miglitol are titrated over two to three weeks to minimize flatulence and other gastrointestinal side effects that lead to discontinuation of these agents. α-glucosidase inhibitors are contraindicated in patients with inflammatory bowel disease, partial intestinal obstruction, a predisposition to intestinal obstruction, colonic ulceration and other gastrointestinal disorders. Dose-dependent hepatotoxicity is associated with this drug class; so liver function tests are carefully monitored in patients receiving higher dosages of these medications (for example, more than 50 mg three times daily). Serum transaminase levels are checked every three months for the first year patients take the medication and periodically thereafter. Intestinal absorbents and digestive enzyme preparations
are not administered with acarbose. However, their major side effects are gas, bloating and diarrhea (Piero et al., 2012d).

2.5.2.5 Thiazolidinediones

The mechanism of action of thiazolidinediones is not fully understood. However, these drugs are known to exert direct effects on the liver and peripheral tissues, which are integrally involved in glucose production and uptake.

Thiazolidinediones are pharmacological ligands for a nuclear receptor known as peroxisome proliferator-activated receptor γ. When activated, this receptor binds to response elements on DNA and alters transcription of various genes that regulate carbohydrate and lipid metabolism. Through this process, thiazolidinediones increase insulin-stimulated glucose uptake in skeletal muscle cells (AACE, 2007).

In type II diabetic patients, thiazolidinedione therapy is associated with a reduction in circulating plasma free fatty acid (FFA) levels and FFA turnover, a shift in fat distribution from visceral to subcutaneous fat storage depots, a decrease in hepatic fat content, and an improvement in peripheral insulin sensitivity. However, no previous study has examined whether the decrease in hepatic fat content and/or plasma FFA concentration is related to improved splanchnic glucose uptake (SGU) following thiazolidinedione treatment in patients with type II diabetes mellitus (Piero et al., 2012d).
The two thiazolidinediones currently available, rosiglitazone and pioglitazone, seem to have similar efficacy on glycemic control. In addition to lowering glycemia, these agents modestly reduce blood pressure, enhance fibrinolysis, and improve endothelial function. Both medications also confer benefits in increasing high-density lipoprotein cholesterol (HDL-C) concentrations and decreasing triglyceride concentrations (AACE, 2007). Pioglitazone or rosiglitazone are usually prescribed as second-line therapy with metformin, or as third-line therapy in combination with sulphonylureas and metformin (known as ‘triple therapy’). However, their use as mono-therapy and in combination with insulin is increasing (Frier & Fisher, 2010).

Adverse effects of thiazolidinediones include weight gain, edema, anemia, and peripheral fractures in women. Weight gain and edema are more commonly seen in patients treated with thiazolidinediones and insulin. The Food and Drug Administration still recommends periodic measurement of hepatic function in patients treated with thiazolidinediones. Thiazolidinediones should not be used in patients with congestive heart failure (AACE, 2007; Frier & Fisher, 2010).

2.5.3 Incretin-based therapies

The secretion of insulin in response to a rise in blood glucose is greater when glucose is given by mouth than by intravenous infusion. In part this is caused by secretion of gut hormones, or incretins, which potentiate glucose induced insulin
secretion. Glucagon-like peptide (GLP-1) is an incretin hormone which stimulates insulin secretion in a glucose-dependant manner. In addition, GLP-1 suppresses glucagon secretion, delays gastric emptying, reduces appetite and encourages weight loss. As GLP-1 is rapidly degraded by enzyme dipeptidyl peptidase 4, inhibitors of this enzyme can be used to prolong its biological effect. The DPP-4 inhibitors or gliptins (sitagliptin, vildagliptin, and saxagliptin) are oral agents which act in this manner (Frier & Fisher, 2010).

Synthetic GLP-1 receptor antagonists with longer therapeutic action include exenatide (synthetic exandin-4) and liraglutide but although they have the advantage of inducing weight loss in most patients, they have to be given daily by subcutaneous injection and may cause nausea. Long-acting GLP-1 receptor antagonists are being evaluated, which will be administered once weekly. Incretin-based therapies are most useful in obese patients and can be used in combination with other oral anti-diabetic agents (Frier & Fisher, 2010). In vitro and in vivo animal models suggest that glucagon-like peptide 1 promotes proliferation and neogenesis from precursor β-cells; however, this has not yet been demonstrated in humans treated with glucagon-like peptide 1 or exenatide (AACE, 2007).
2.5.4 Herbal management of diabetes mellitus

The World Health Organization (WHO) estimates that up to 80% of the world’s population, mostly in developing countries, relies on traditional medicine practices for its health care needs. This is particularly true of the poorer sections of the population in developing countries because natural remedies are not only cheaper than modern medicines, but are often the only medicines available in remote rural regions (Musila, Kisangau & Muema, 2004).

A notable number of modern drugs have been synthesized from these natural medicinal plants. These drugs are based on the indigenous medicinal information of plants. This natural source has been used to cure various diseases throughout the world. Actually, plants have great diversity of bioactive compounds and it is an indication which makes plants a prosperous source of different types of drugs (Mahmood, Mahmood & Qureshi, 2012).

In Kenya, traditional medicine continues to play a major role in Primary Health Care (PHC). More than 70% of the Kenyan population relies on traditional medicine as its primary source of health care, while more than 90% use medicinal plants, at one time or another. It is more accessible than modern health facilities for most of the population in the country. It is relatively inexpensive, locally available, and usually accepted by the local communities as comparable to modern conventional medicine (Musila, Kisangau & Muema, 2004).
Presently, there is growing interest in herbal remedies due to the side effects associated with the oral hypoglycemic agents (therapeutic agent) for the treatment of diabetes mellitus. Traditional herbal medicines play an important role in the management of diabetes mellitus. Several species of herbal drugs have been described in the scientific and popular literature as having antidiabetic activity. Due to their perceived effectiveness, fewer side effects in clinical experience and relatively low costs, herbal drugs are prescribed (Rao et al, 2010).

More than 400 traditional plant treatments for diabetes mellitus have been recorded, but only a small number of these have received scientific and medical evaluation to assess their efficacy. Traditional treatments have mostly disappeared in occidental 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 underdeveloped regions. A hypoglycemic action from some treatments has been confirmed in animal models and non-insulin-dependent diabetic patients, and various hypoglycemic compounds have been identified (Bailey & Day, 1989).

In diabetes, some herbal alternatives are proven to provide symptomatic relief and assist in the prevention of the secondary complication of the disease. Some herbs have also been proven to help in regeneration of β-cells and in overcoming resistance. In addition to maintaining normal blood sugar level, some herbs are
also reported to possess antioxidant activity and cholesterol lowering action (Pandey et al, 2011).

Biological actions of the plant products used as alternative medicines to treat diabetes are related to their chemical composition. Herbal products or plant products are rich in phenolic compounds, flavonoids, terpenoids, coumarins, and other constituents which show reduction in blood glucose levels (Rao et al, 2010). Traditional antidiabetic plants might provide a useful source of new oral hypoglycemic compounds for development as pharmaceutical entities, or as simple dietary adjuncts to existing therapies (Bailey & Day, 1989). The following are some of the most common antidiabetic plants used in the tradition medicine:

2.5.4.1 *Allium cepa* and *Allium sativum*

Allium species such as onions (*Allium cepa*) and garlic (*Allium sativum*) are used as foodstuff, condiment, flavoring, and folk medicine. Onion and garlic are rich in sulphur containing compounds mainly in the form of cysteine derivatives, like S-alkyl cysteine sulphoxide which is decomposed by the enzyme alliinase into a variety of volatile compounds such as thiosulphinates and poly-sulfides during extraction (El-Demerdash, Yousef & Abou El-Naga, 2005).

Onion has a globose bulb that is an underground part of the stem and is so often treated as a single household vegetable. It has been used medicinally for hundreds
of years (Ozougwu, 2011). The antidiabetic effect of onion was reported by several studies using experimental animal models as well as human diabetes patients (Mathew & Augusti, 1975; El-Demerdash, Yousef & Abou El-Naga, 2005; Kook, Kim & Choi, 2009; Ozougwu, 2011; Ogunmodede et al, 2012).

Garlic is one of the most popular herbs used worldwide to reduce various risk factors associated with cardiovascular diseases (Thomson et al, 2007). It contains a higher concentration of sulfur compounds than any other Allium species. The sulfur compounds are responsible both for garlic’s pungent odor and many of its medicinal effects (Londhe et al, 2011). Garlic principle active agent appears to be allicin, a sulfur-containing compound that with its breakdown products gives garlic its characteristic odor. Allicin is formed enzymatically from an odorless precursor, alliin, when garlic cloves are mechanically disrupted (Phil, Khan & Ashraf, 2011).

In diabetic patients, it was reported that garlic oil can correct hyperglycemia. In addition, a precursor of various allyl sulfide constituents of garlic oil, S-allylcysteine sulfoxide (alliin), was shown to have a hypoglycemic effect similar to that of glibenclamide. Garlic has been found to be effective in lowering serum glucose levels in STZ-induced as well as alloxan-induced diabetic rats and mice through increased insulin secretion and increased insulin sensitivity (Liu et al, 2006; Khayatnouri, Bahari & Safarmashaei, 2011; Londhe et al, 2011). Phil,
Khan & Ashraf (2011) reported that garlic has antidiabetic effect on human diabetic patients. Prepared garlic tablets of different doses given to type II diabetes patients showed significant decrease in fasting blood sugar and HbA1C in both dose dependent and duration dependent manner.

2.5.4.2 *Nigella sativa*

*Nigella sativa* is a herbaceous plant growing to about 20-30 cm in height, commonly known as black seed because of the small triangular black seeds it generates. It has been consumed for more than 2000 years, is used extensively in the traditional medicine of many southern Mediterranean and Middle Eastern countries, and has been shown to produce multi-systemic beneficial actions (Benhaddou-Andaloussi et al, 2011). The ethanol extract of *N. sativa* showed good anti-diabetic activity on alloxan induced diabetic rats (Khan et al, 2013).

Benhaddou-Andaloussi et al (2008) have demonstrated that ethanol extract of *N. sativa* seed exhibits the remarkable ability *in vitro* to concomitantly increase insulin secretion, induce proliferation of pancreatic β-cells, and stimulate glucose uptake in skeletal muscle and fat cells. In addition the aqueous extract of *N. sativa* was reported to directly inhibit the electronegic intestinal absorption of glucose *in vitro* and improve glucose tolerance and body weights in rats after chronic oral administration *in vivo* (Paarakh, 2010). In human *Nigella sativa* at a dose of 2...
g/day caused significant reductions in FBG, 2hPG, and HbA1c without significant change in body weight (Bamosa et al, 2010).

2.5.4.3 Aloe vera

*Aloe vera* is a cactus-like plant with green dagger-shaped leaves that are fleshy, tapering, spiny, marginated and filled with a clear viscous gel (Saif-Ur-Rehman et al, 2011). It grows in arid climates and is widely distributed in Africa, India and other arid areas (Kavishankar et al, 2011). Cosmetic and medicinal products are made from the mucilaginous tissue in the centre of the *Aloe vera* called *Aloe vera* gel. The peripheral bundle of sheath cells produce intensely bitter, yellow latex, commonly termed aloe juice, or sap or aloes (Yagi et al, 2009). The name was derived from the Arabic ‘alloeh’ meaning ‘bitter’, because of the bitter liquid found in the leaves. *A. vera* has long been used all over the world for its various medicinal properties (Jafri et al, 2011).

The antidiabetic effect of *A. vera* was confirmed in experimental animals (Gupta et al, 2011; Kavishankar et al, 2011; Saif-Ur-Rehman et al, 2011) as well as in human diabetes patients (Yongchaiyudha et al, 1996; Yagi et al, 2009).

2.5.4.4 Azadirachta indica

*A. indica* is commonly known as neem. It has been long used as a treatment for diabetes. Aqueous leaves extracts of neem significantly decreases blood sugar
level and prevents adrenaline as well as glucose-induced hyperglycemia (Pandey et al., 2011). The aqueous leaf extract orally administered decreased blood glucose levels in STZ-induced diabetes rats. Aqueous fruit extracts of neem reduced the normal blood glucose levels in normoglycemic rabbits (Rao, Madhuri & Prasad, 2012). In addition Patil et al (2013) reported that alcohol extract of neem root bark has showed anti-hyperglycemia activity in alloxan induced diabetes rats.

2.5.4.5 Gymnema sylvestre

Commonly known as Gurmar, has long been used as a treatment for diabetes (Pandey et al., 2011). In a study of type II diabetes, 22 patients were given 400 mg/kg body weight of the aqueous leaf extracts of Gymnema sylvestre daily for 18-20 months as a supplement to the conventional oral drugs. During Gymnema sylvestre supplementation the patients showed a significant reduction in blood glucose, glycosylated hemoglobin and glycosylated plasma proteins, and conventional drug dosage could be decreased.

Five of the 22 diabetic patients were able to discontinue their conventional drug and maintain their blood glucose homeostasis with Gymnema sylvestre alone. These data suggest that the β-cells may be regenerated/ repaired in type II diabetic patients on Gymnema sylvestre supplementation. This is supported by the appearance of raised insulin levels in the serum of patients after Gymnema sylvestre supplementation (Baskaran et al, 1990).
2.5.4.6 *Momordica charantia*

Also known as bitter melon, it is a tropical plant that is widely cultivated in Asia, India, South America and East Africa for its intensely bitter fruits that are commonly used in cooking and as natural remedy for treating diabetes (Joseph & Jini, 2013). The blood sugar lowering action of fresh juice or unripe fruit has been established in animal experimental models as well as human clinical trials (Welihinda *et al.*, 1986; Srivastava *et al.*, 1993; Chowdhury *et al.*, 2012).

2.6 Plants of this study

2.6.1 *Kleinia squarrosa* (locally known as *Mung'endya nthenge*).

This plant has been in use by traditional health practitioners to treat different human diseases such as jaundice, stomach pain, asthma, syphilis, edema, malaria, and for women with menstruation problems (Musila, Kisangau & Muema, 2004). The plant has also been in use successfully in the management of diabetes mellitus in some parts of Kenya. Its aqueous stem bark extract given through intra-peritoneal route exhibited remarkable hypoglycemic properties in alloxan induced diabetic mice (Murugi *et al.*, 2012).

2.6.2 *Acacia nilotica* (locally known as *Musemei*).

*Acacia nilotica* is a common, medium sized tree, belonging to the family Mimosaceae and used traditionally to treat Gonorrhea, chest pain, cough (Musila, Kisangau & Muema, 2004). The pods of *A. nilotica* are fed to cattle to increase
the milk yield and also used ethno-medicinally for the treatment of skin diseases, stomach ache, malaria, sore throat as well as for tooth problems (Naqvi et al, 2011). Sultana, Anwar & Przybylski (2007) reported that bark extract of Acacia nilotica exhibits antioxidant properties.

Water and methanol extracts of different parts of Acacia nilotica (leaves, stem and bark) exhibited significant anti-bacterial activity against both gram positive and gram negative pathogens, and also showed good anti fungal activity against some fungal strains (Naqvi et al, 2011). The anti-diabetes effect of Acacia nilotica has been investigated in Pakistan and it has shown good hypoglycemic and hypolipidemic activity (Ahmad et al, 2008).

2.6.3 Aloe secundiflora (locally known as Kiluma)

Aloe species have been valued since prehistoric times as medicine for the treatment of burns, wound infections and other skin problems. The latest review of some aloe species shows that they have antibacterial, antifungal, antiviral, anticancer; anti-diabetes and immunomodulatory properties (Waihenya et al, 2002).

Aloe secundiflora is traditionally used to treat painful diaphragm, malaria, edema, nose bleeding, diarrhea; and typhoid fever (Musila, Kisangau & Muema, 2004). Mariita et al (2011) investigated *in vitro* antimicrobial activity of methanol
extract of *Aloe secundiflora* and found that it exhibits strong antibacterial activities against a number of Microorganisms including four strains of mycobacteria (*M. tuberculosis, M. kansasii, M. fortuitum* and *M. smegmatis*), *Salmonella typhi, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, and Klebsiella pneumoniae*.

It was observed that crude extracts of *Aloe secundiflora* mature leaves showed antimicrobial effect on *Candida albicans* a fungus that is the second most cause of vaginal irritation or vaginitis and can also occur in male genital organ (Msoffe & Mbilu, 2009). *Aloe secundiflora* has also ethno-veterinary properties. It was reported that the crude extract of the plant delayed the occurrence of the clinical signs and reduced severity of the disease in experimentally infected chickens with *Salmonella gallinarum* (bacteria that causes Fowl typhoid) (Waihenya *et al.*, 2002).

### 2.6.4 *Zanha africana* (locally known as *Mukolekya*)

*Zanha africana* is a plant remedy used by traditional health practitioners for treatment of many diseases such as diarrhea, typhoid fever, pneumonia, scabies, nose bleeding and to prevent and stop bleeding for women. The plant is also frequently used for the treatment of sexually transmitted diseases especially gonorrhea (Musila, Kisangau & Muema, 2004).
The antibacterial activity of both water and organic extracts of this plant has been demonstrated with the methanol extracts exhibiting the greatest antibacterial activity (Kambizi & Afolayan, 2001). Fabry, Okemo & Ansorg (1996) reported that the plant has considerable antifungal activity against candida species. It was also reported that the crude extracts of this plant caused substantial growth inhibition for Trypanosoma brucei (Nibret et al, 2010).

2.6.5 Fuerstia africana (Locally known as kalaku)

It belongs to the family Lamiaceae and used traditionally to treat Malaria, pneumonia, ulcers, infertility and stomach pain (Musila, Kisangau & Muema, 2004). The organic extracts of F. africana exhibits high antimicrobial activity against different strains of bacteria, while its methanol extract exhibits highest antimicrobial activity against Klebsiella pneumonia (Matu et al, 2012). A recent research found that the methanol extracts of F. africana have good anti-plasmodium activity in vitro and moderate anti-malarial activity in vivo (Muthaura et al, 2007).
CHAPTER THREE
MATERIALS AND METHODS

3.1 Plant samples
The plants used in this study were collected from their natural habitats in various parts of Machakos County, Kenya on the basis of ethnobotanical information. An acknowledged authority in taxonomy authenticated the botanical identity of the plants and a voucher specimen was deposited at the National Museums of Kenya Herbarium, Nairobi. The plants collected and studied were Acacia nilotica, Fuerstia africana, Aloe secundiflora, Kleinia squarrosa, and Zanha africana. A traditional medical practitioner provided the information on which plant to collect, what part to collect, and the precise locality where it grows.

3.2 Initial processing of the plants
The parts of the plants collected were, stem barks and leaves. The stems were harvested and their barks peeled off while still fresh, cut into small pieces and then dried at room temperature for 1 month. Leaves were collected while green and dried in the same way. The stem barks and the leaves were separately ground when completely dry using an electric mill. The powdered plant materials were labeled and kept at room temperature away from direct sunlight in closed dry plastic bags.
3.3 Preparation of the aqueous extracts

One hundred grams of each powdered plant material was extracted in 1 liter distilled-deionized water at 60°C for 6 hour. The mixture was left to cool at room temperature and then decanted into dry clean conical flask through folded cotton gauze stuffed into a funnel. The decanted extract was then filtered using filter papers under vacuum pump. The filtrate was then freeze-dried for 72 hour. The freeze-dried powder was then weighed and stored in airtight container at -20°C until used for bioassay.

3.4 Experimental animals

The study used male Swiss White Albino mice (3-4 weeks old) that weighed 23-27g with a mean weight of 25g. These were bred in the Animal house at the Department of Biochemistry and Biotechnology of Kenyatta University. 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 libitum. The experimental protocols and procedures used in this study were approved by the Ethics Committee for the Care and Use of Laboratory Animals of Kenyatta University, Kenya.

3.5 Induction of hyperglycemia

Hyperglycemia was induced experimentally by a single intraperitoneal administration of 186.9 mg/kg body weight of a freshly prepared 10% alloxan
monohydrate (2,4,5,6 tetraoxypyrimidine; 5-6-dioxyuracil) obtained from Sigma
(Steinhein, Switzerland) (Karau et al, 2012).

Forty-eight hours after alloxan administration, blood glucose level was measured
using a glucometer. Mice with blood glucose levels above 2000 mg/L were
considered diabetic and used in this study. Prior to initiation of this experiment,
the animals were fasted for 8-12 hours (Szkudelski, 2001) but allowed free access
to water until the end of this experiment.

3.6 Experimental design

For both intraperitoneal and oral route of drug administration, the experimental
mice were randomly divided into seven groups of five animals each. Group Ia and
Group Ib consisted of normal mice administered with 0.1ml physiological saline
intraperitoneally and orally, respectively; Group IIa and Group IIb consisted of
alloxan induced diabetic mice administered with 0.1ml physiological saline
intraperitoneally and orally, respectively; Group IIIa consisted of alloxan induced
diabetic mice intraperitoneally administered with 0.025 insulin units (0.25 insulin
units in 1 ml) (1 IU/kg body weight) in 0.1ml physiological saline; Group IIIb
consisted of alloxan induced diabetic mice orally administered with 0.075 mg
glibenclamide (0.75 mg in 1 ml) (3 mg/kg body weight) in 0.1 ml physiological
saline.
Group IVa and Group IVb consisted of alloxan induced diabetic mice administered with 1.25 mg extract (12.5 mg in 1 ml physiological saline) (50 mg/kg body weight) in 0.1 ml physiological saline intraperitoneally and orally, respectively; Group Va and Group Vb consisted of alloxan induced diabetic mice administered with 2.5 mg extract (25 mg extract in 1 ml physiological saline) (100 mg/kg body weight) in 0.1 ml physiological saline intraperitoneally and orally, respectively; Group VIa and Group Vlb consisted of alloxan induced diabetic mice administered with 5 mg extract (50 mg extract in 1 ml physiological saline) (200 mg/kg body weight) in 0.1 ml physiological saline intraperitoneally and orally, respectively; Group VIIa and Group VIIb consisted of alloxan induced diabetic mice administered with 7.5 mg extract (75 mg extract in 1 ml physiological saline) (300 mg/kg body weight) in 0.1 ml physiological saline intraperitoneally and orally, respectively. Either 0.1 ml of insulin or glibenclamide or the plant extract solution was administered either intraperitoneally or orally to each experimental mouse.

3.7 Blood sampling and glucose determination

Blood sampling was done by sterilizing the tail with 70% alcohol and then nipping the tail at the start of the experiment and repeated after 1, 2, 3, 4, 6 and 24 hours. Bleeding was enhanced by gently “milking” the tail from the body towards the tip. After the operation, the tips of the tail were sterilized by swabbing with
70% ethanol. The blood glucose levels were determined with a glucose analyser model (Hypogaurd, Woodbridge, England).

3.8 In vivo single dose toxicity test

The mice were randomly divided into four different groups of five mice each. Group I and II consisted of untreated control mice intraperitoneally and orally, respectively, administered daily for 28 days with 0.1ml physiological saline. Group III and IV consisted of normal mice intraperitoneally and orally administered daily for 28 days with 25 mg (1g/kg body weight) of the plant extracts in 0.1ml physiological saline. During this period, mice were allowed free access to mice pellet and water and observed for any signs of general illness, change in behavior and mortality. At the end of 28 days, the mice were sacrificed.

3.9 Determination of body and organ weight

The body weight of each mouse was assessed after every seven days during the dosing period up to and including the 28th day and the day of sacrifice (day zero, 7, 14, 21, 28). On the day of sacrifice, all the animals were euthanized using isoflurane as an inhalant anaesthesia and blood samples were drawn from the heart of each sacrificed mouse. The blood samples were collected in plastic test tubes and divided into two portions. One portion was used for determination of hematological parameters. The other portion was allowed to stand for 3 hours to ensure complete clotting. The clotted blood samples were centrifuged at 3000 rpm.
for 10 min and clear serum samples were aspirated off and stored frozen at -20°C for metabolite and enzyme assays. The liver, kidney, heart, lungs, spleen, intestine, brain and testis were carefully dissected out, weighed and preserved in 10% neutral buffered formalin for histological analysis.

3.10 Determination of hematological parameters

Blood parameters and indices were determined using standard protocols (Jain, 1986). Red blood cells count (RBC), white blood cells count (WBC), hemoglobin (Hb), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), packed cell volume (PCV), mean corpuscular volume (MCV) and platelets (PLT) were determined in whole blood with EDTA anticoagulant using the Coulter Counter System (Beckman Coulter®, ThermoFisher, UK).

Differential white blood cell count for neutrophils, lymphocytes, eosinophils, basophils and monocytes were determined from giemsa stained blood films using a hemocytometer (Jain, 1986). Air-dried thin blood films stained with giemsa stain were examined microscopically using magnification 400 and 1000 for differential WBC counts and cell morphology, respectively.
3.11 Determination of biochemical parameters

Determination of biochemical parameters was done in the Department of Laboratory Medicine, Kenyatta National Hospital. The biochemical parameters determined on the sera specimen (see section 2.9) using the Olympus 640 Chemistry Auto-Analyzer were Aspartate aminotransferase (AST), Alanine transaminase (ALT), Alkaline phosphatase (ALP), Gamma-glutamyl transpeptidase (GGT), Lactate dehydrogenase (LDH), Creatine Kinase (CK), \( \alpha \)-Amylase (\( \alpha \)-AMYL), Total bilirubin (T-BIL), Direct bilirubin (D-BIL), Urea and Creatinine. All reagents for the machine were commercially prepared to fit the required volumes and concentrations. The reagents were in specific containers referred to as reagent cartridges.

The reagent cartridges were bar coded for the identification by the machine. The machine was programmed for the selected tests for each sample. The sample sectors were then placed into the autoloader assembly. A number of events that occurred simultaneously were performed automatically under the direct control of the instrument microprocessor. All the assays were performed based on the standard operating procedures (SOPs) written and maintained in the Department of Laboratory Medicine, Kenyatta National Hospital.
3.11.1 Determination of serum levels of aspartate aminotransferase (AST)

The method used is described by Henry et al (1960). AST reagent was used to measure AST level by an enzymatic rate method. In the reaction, AST catalyzed the reversible transamination of L-aspartate and α-ketoglutarate to oxaloacetate and L-glutamate. The oxaloacetate was then reduced to malate in the presence of malate dehydrogenase (MDH) with concurrent oxidation of reduced β-nicotinamide adenine dinucleotide (NAD). The ratio of the sample to reagent was 1 part sample to 11 parts reagents (23μL: 253μL reagent). The absorbency was measured at 340 nm and its change was directly proportional to the levels of AST. The machine calculated and expressed the level in IU/L. The reaction took place at 37°C for three minutes. The principal of the reaction is as follows:

\[
2\text{-oxoglutarate} + \text{L-aspartate} \rightarrow_{\text{AST}} \text{L-glutamate} + \text{Oxaloacetate}
\]

\[
\text{Oxaloacetate} + \text{NADH} + \text{H}^+ \rightarrow_{\text{MDH}} \text{Malate} + \text{NAD}^+
\]

3.11.2 Determination of serum levels of alanine aminotransferase (ALT)

The method used is described by Henry et al (1960). The ALT reagent was used to measure ALT level by an enzymatic rate method. In the reaction, the ALT catalyzes the reversible transamination of L-alanine and α-ketoglutarate to pyruvate and L-glutamate. The pyruvate is then reduced to lactate in the presence of Lactate dehydrogenase (LDH) with concurrent oxidation of β-Nicotinamide
Adenine Dinucleotide (reduced form) (NADH) to β-Nicotinamide Adenine Dinucleotide (oxidized form) (NAD⁺). 

Pyridoxal-5-phosphate was required in this reaction as a cofactor for the transaminase activity by binding to the enzyme using Schiff-base linkage. The ratio of the sample to reagent was 1 part sample to 11 parts reagents (23 μL: 253 μL reagent). The absorbance was measured at 340 nm and its change was directly proportional to the level of ALT. The machine calculated and expressed the level in IU/L. The reaction took place at 37°C for 3 min. The principal of the reaction is as follows:

\[
\begin{align*}
2\text{-oxoglutarate} + \text{L-alanine} & \xrightleftharpoons{\text{ALT}} \text{L-glutamate} + \text{pyruvate} \\
\text{Pyruvate} + \text{NADH} + \text{H}^+ & \xrightleftharpoons{\text{LDH}} \text{Lactate} + \text{NAD}^+
\end{align*}
\]

3.11.3 Determination of serum levels of alkaline phosphatase (ALP)

The ALP reagent was used to measure ALP level by kinetic method using a 2-amino-2-methyl-1-propanol (AMP) buffer. In the reaction, ALP catalyzed the hydrolysis of the colorless organic phosphate ester substrate (p-nitrophenylphosphate) to the yellow colored product (p-nitrophenol and phosphate). This reaction occurred at alkaline pH of 10.3. The ratio of the sample to reagent was 1 part sample to 50 parts reagents (5 μL: 250 μL reagent). The absorbance was measured at 410 nm and this change was directly proportional to
the level of ALP. The machine calculated and expressed the level in IU/L. The reaction took place at 37°C for three minutes (International Federation of Clinical Chemistry, 1983). The principal of the reaction is as follows:

\[ 4\text{-NPP} + \text{H}_2\text{O} \xrightarrow{\text{ALP}} 4\text{-NPO} + \text{Phosphate} \]

3.11.4 Determination of serum levels of \( \gamma \)-glutamyl transferase (\( \gamma \)-GT)
GGT reagent was used to measure \( \gamma \)-glutamyltransferase level by an enzymatic kinetic UV rate method. In the reaction, \( \gamma \)-glutamyltransferase catalyzed the transfer of the glutamyl group from the substrate to glycyglycine forming glutamylglycylglycine and 5-amino-2-nitrobenzoate. 5\( \mu \)l of the sample was reacted with 200\( \mu \)l of the reagent. The rate of formation of 5-amino-2-nitrobenzoate was proportional to the level of GGT present in the sample and was measured kinetically at 405nm. The level was calculated and expressed in U/L. The reaction took place at 37°C for three minutes. The principal of the reaction is as follows:

\[ \text{L-} \gamma\text{-glutamyl-3-carboxy-4-nitroanilide} + \text{glycylglycine} \xrightarrow{\text{GT}} \text{L-} \gamma\text{-glutamylglycylglycine} + 5\text{-amino-2-nitrobenzoate} \]

3.11.5 Determination of serum levels of lactate dehydrogenase (LDH)
This was an enzymatic kinetic UV test for the quantitative determination of LDH. In the reaction, LDH catalyzed the oxidation of lactate to pyruvate coupled with the reduction of NAD\(^+\) to NADH. 2 \( \mu \)L of sample was reacted with 40 \( \mu \)L of
reagent and the change in absorbance due to reduction of NAD was monitored at 340nm. This change was directly proportional to the concentration of LDH in the sample and was used to calculate and express concentration in U/L. The reaction took place at 37°C for three and half minutes. The pH optimum for lactate-to-pyruvate (L → P) reaction is 8.8 to 9.8. The principal of the reaction is as follows:

\[
\text{Lactate} + \text{NAD}^+ \xrightarrow{LDH} \text{Pyruvate} + \text{NADH} + \text{H}^+
\]

3.11.6 Determination of serum levels of creatine kinase (CK)
The method used is described as Oliver and Rosalki method based on the primary reaction that is catalyzed by CK resulting in production of creatine and ATP. The ATP produced in the primary reaction is then employed in a coupled enzymatic glucose assay employing hexokinase (HK) and glucose-6-phosphate dehydrogenase (G6PD). The ratio of the sample to reagent was 1 part sample to 50 parts reagents (5μL: 250μL reagent). The machine calculated and expressed the level in IU/L. The reaction took place at 37°C for three minutes. The production of NADPH in the indicator reaction is monitored at 340 nm and is related to CK level within the patient specimen (Rosalki, 1967). The principal of the reaction is as follows:

\[
\text{ADP} + \text{Creatinine phosphate} \xrightarrow{CK} \text{Creatinine} + \text{ATP}
\]

\[
\text{ATP} + \text{Glucose} \xrightarrow{HK} \text{ADP} + \text{Glucose-6-phosphate}
\]
Glucose-6-phosphate+\text{NAD}^+ \xrightarrow{G6PD} 6\text{-Phosphogluconate} + \text{NADH} + \text{H}^+$

3.11.7 Determination of serum levels of $\alpha$-amylase ($\alpha$-AMYL)

Amylase reagent was used to measure the concentration of amylase by a kinetic colour method using Olympus Autoanalyzer. In the reaction, 2-chloro-4-nitrophenyl-$\alpha$-D-maltotrioside (CNPG$_3$) substrate reacted with amylase in the serum to release 2-chloro-4-nitrophenol (CNP) from the substrate which was directly proportional to the concentration of amylase in the sample. Three (3) $\mu$L of sample was reacted with 300 $\mu$L of reagent and the change in absorbance was monitored at 340 nm, due to reduction of NAD.

This change was directly proportional to the concentration of AMY in the sample and was used to calculate and express concentration in U/L. The reaction took place at 37°C for three and half minutes. The principal of the reaction is as follows:

$$\text{CNPG}_3 + \text{H}_2\text{O} \xrightarrow{\text{Amylase}} \text{CNP} + \text{Maltotriose}$$

3.11.8 Determination of serum levels of total bilirubin (T-BIL)

Total bilirubin reagent was used to measure BIL-T concentration by timed end point reaction. In the presence of hydrochloric acid (HCL), T-BIL was coupled with diazotized Sulfanilic acid (pH 1.4) forming azobilirubin. The chemistry analyzer automatically aliquoted 9 $\mu$L sample and 120 $\mu$L BIL-T reagents then
photometrically measured azobilirubin whose colour intensity was directly proportional to the T-BIL concentration. Determination took place at 552 nm for two minutes at 37°C. The analyzer automatically calculated the T-BIL concentration and expressed it in μmol/L. The principal of the reaction is as follows:

\[
\text{Sulfanilic acid} + \text{NaNO}_2 \rightarrow^{\text{HCl}} \text{Diazotized Sulfanilic acid}
\]

\[
\text{Bilirubin} + \text{Diazotized Sulfanilic acid} \rightarrow^{\text{pH} 1.4} \text{Azobilirubin}
\]

3.11.9 Determination of serum levels of direct bilirubin (D-BIL)

Conjugated and direct bilirubin reagent was used to measure D-BIL concentration by timed end point reaction. In the presence of hydrochloric acid (HCl), D-BIL was coupled with diazotized Sulfanilic acid (pH 1.4) forming azobilirubin. The chemistry analyzer automatically aliquoted 9 μl sample and 120μl D-BIL reagent then photometrically measured azobilirubin whose colour intensity was directly proportional to the D-BIL concentration. Determination took place at 552 nm for two minutes at 37°C. The analyzer automatically calculated the D-BIL concentration and expressed it in μmol/L. The principal of the reaction is as follows:

\[
\text{Sulfanilic acid} + \text{NaNO}_2 \rightarrow^{\text{HCl}} \text{Diazotized Sulfanilic acid}
\]

\[
\text{Bilirubin} + \text{Diazotized Sulfanilic acid} \rightarrow^{\text{pH} 1.4} \text{Azobilirubin}
\]
3.11.10 Determination of serum levels of urea

The method used is described by Tiffany et al (1972). The BUN reagent was used to measure the concentration of urea by an enzymatic rate method. In the reaction, urea was hydrolyzed by urease to ammonia and carbon dioxide. Glutamate dehydrogenase (GIDH) catalyzed the condensation of ammonia and α-ketoglutarate to glutamate with concomitant oxidation of reduced β-Nicotinamide Adenine Dinucleotide (NADH) to β-Nicotinamide Adenine Dinucleotide (NAD⁺). The ratio of the sample to reagent was 1 part sample to 100 parts reagents (3μL: 300μL reagent). The absorbance was measured at 340 nm and this change was directly proportional to the concentration urea. The machine calculated and expressed the concentration in μM. The reaction took place at 37°C for one minute. The principal of the reaction is as follows:

\[
\text{Urea} + \text{H}_2\text{O} \xrightarrow{\text{Urease}} 2\text{NH}_3 + \text{CO}_2
\]

\[
\text{NH}_3 + \alpha\text{-ketoglutarate} + \text{NADH} \xrightarrow{\text{GIDH}} \text{L-Glutamate} + \text{NAD}^+.
\]

3.11.11 Determination of serum levels of creatinine (CREAT)

Creatinine reagent was used to measure CREAT concentration by a modified rate Jaffe. In the reaction, creatinine reacted with picric acid in alkaline solution to form creatinine-picric acid complex. The analyzer automatically proportioned 15 μl sample and 250 μl creatinine reagent into the reaction cuvette. The rate of colour formation was monitored and increase in absorbance determined at 512 nm.
for two minutes at 37°C. The analyzer automatically calculated the CREAT concentration and expressed it in mmol/L. The principal of the reaction is as follows:

\[
\text{Creatinine + Picric acid} \xrightarrow{\text{heat}} \text{Creatinine-picric acid (yellow-red) complex}
\]

3.12 Histopathology

The formalin fixed tissues (see section 2.9) were processed using standard histopathology protocol and stained with haematoxylin and eosin for microscopy. The stained tissues were cover slipped with DPX, dried and examined microscopically for any pathological changes (Baker, Silverton & Luckcock, 1989).

3.13 Phytochemical Screening

Phytochemical screening for alkaloids, saponins, flavonoids, total phenols and tannins were conducted on the aqueous extract both qualitatively and quantitatively using standard procedures.

3.13.1 Qualitative Analysis on Phytochemical Constituents

3.13.1.1 Alkaloids

For the alkaloids, to the 2 ml methanolic filtrate of the extract, 1.5 ml of 1% HCl was added. After heating the solution in water bath, 6 drops of Mayors
reagents/Wagner’s reagent/Dragendorff reagent was added. Formation of Orange precipitate was an indication of the presence of alkaloids (Krishnaiah et al, 2009).

3.13.1.2 Saponins

Aqueous extract of 2 g extract of the plant material was made and subjected to frothing test. Frothing persistence indicated presence of saponins (Houghton & Raman, 1998). Latter the froth was mixed with few drops of olive oil. Formation of emulsion indicates presence of saponins (Houghton & Raman, 1998).

3.13.1.3 Flavonoids

2 g of plant material was extracted in 10 ml alcohol. To 2 ml filtrate few drops of concentrated HCl followed by 0.5 g of magnesium turnings was added. After 3 minutes magenta red or pink color indicated the presence of flavonoids (Parekh & Chanda, 2007).

3.13.1.4 Total phenols

The phenolics were determined as follows; to 2 ml of alcoholic or aqueous extract, 1 ml of 1% ferric chloride solution was added. Blue or green color indicates phenols (Houghton & Raman, 1998).
3.13.1.5 Tannins

To 2 ml of aqueous extract, 2 ml of 5% FeCl₃ was added. Formation of yellow brown precipitate indicated that tannins are present (Parekh & Chanda, 2007).

3.13.2 Quantitative analysis on phytochemical constituents

The amounts of various phytochemicals screened were determined as follows:

3.13.2.1 Alkaloids

The alkaloid content was determined gravimetrically (Harborne, 1998) with some modifications. 1.25 g of each sample was first defatted three times using hexane followed by extraction using 50 ml of 10% acetic acid in ethanol. The mixtures were shaken well, covered and allowed to stand for 4 h. The mixtures were then filtered and the extracts concentrated in water bath until 1/4 of the original volume was attained. Concentrated ammonium hydroxide was then added drop wise in order to precipitate the alkaloids. Pre-weighed filter papers were used to filter off the precipitates which were then washed with 1% ammonium hydroxide solution. The filter papers containing the precipitates were dried on an oven at 60°C for 30 minutes, transferred into desiccator to cool and then reweighed until constant weights were obtained. The weights of the alkaloid were determined by weight differences of the filter. The experiments were replicated twice for each sample and the readings recorded as the average of the two replicates.
3.13.2.2 Saponins

Quantification of saponins was done according to the method of Obadoni & Ochuko (2001) with some modifications. 1.5 g each of the samples was extracted with methanol in Soxhlet apparatus for eight hours. The methanolic extracts were evaporated under reduced pressure to afford crude methanolic extracts which were partitioned between hexane and water in separating funnels. The aqueous layers were then extracted with diethyl ether. The aqueous layers were recovered while the diethyl ether layers were discarded and the purification process repeated. The aqueous layers were further partitioned with n-butanol three times in each case. The combined butanol extracts were washed twice with 15 ml of 5% sodium chloride and then evaporated in vacuo to yield crude saponins.

3.13.2.3 Flavonoids

The total flavonoid concentration was measured by the aluminum chloride colorimetric assay (Marinova, Ribaurova & Atanassova, 2005). The extract (0.15 g) was added to a 10 mL volumetric flask containing 4 mL of double distilled water. To the above mixture, 0.3 mL of 5% NaNO₂ was added. After 5 minutes, 0.3 mL of 10% AlCl₃ was added. After 6 minutes, 2 mL of 1 M NaOH was added and the total volume was made up to 10 mL with double distilled water. The solution was mixed well and the absorbance was measured at 510 nm against a blank. The flavonoid content was determined using quercetin as standard.
3.13.2.4 Total phenols

The total phenolic content was determined using Folin-Ciocalteau reagent and gallic acid as the standard according to the method by Rasineni, Siddavattam & Reddy (2008). 500 mg of the aqueous plants extract was weighed and homogenized in 10ml of n-hexane. The homogenate was centrifuged at 10,000 x g for 20 minutes and the supernatant was used in the determination of total phenols as follows. 0.5 ml of Folin-Ciocalteau 2 N reagent was added to 2.5 ml of the supernatant and then 2 ml of 10% sodium carbonate in ethanol. The mixture was incubated for 5 minutes at 20°C and then the absorbance read in triplicates at wavelength of 750 nm.

The gallic acid standard was prepared by dissolving 100 mg of Gallic acid (SD’S Lab-Chem Industry Bombay-India), in 100 ml of distilled water to make the standard stock. This was serially diluted into the working range of; 0.5, 1, 2, 4, 8 and 16 mg/100ml. To each 2.5 ml of the serially diluted standard, 2 ml of the 10% w/v sodium carbonate solution and 0.5 ml of Folin-Ciocalteau 2 N were added and incubated for 5 minutes. The absorbance was read at 750 nm before 15 minutes. The mixture of distilled water, 10% sodium carbonate and Folin-Ciocalteau 2 N was used as the blank. The absorbances were read from UV-VIS spectrophotometer (UV-1700 Pharmaspec, UV-VIS Spectrophotometer, Shimadzu Japan). The total phenol content was expressed as mg/g dry weight gallic acid equivalent.
3.13.2.5 Tannins

The tannins were determined as follows; 2g of plant powder was extracted thrice in 70% acetone. After centrifuging the sample supernatant was removed. Different aliquots were taken and final volume was adjusted to 3 ml using distilled water. After vortexing the solutions were mixed with 1 ml of 0.016M K$_3$Fe (CN)$_6$, followed by 1 ml of 0.02 M FeCl$_3$ in 0.10 M HCl. Vortexing was repeated and the tubes were kept as such for 15 min. 5 ml of stabilizer (3:1:1 ratio of water, H$_3$PO$_4$ and 1% gum arabic) was added followed by revortexing. Absorbance was measured at 700 nm against blank. Standard curve was plotted using various concentrations of 0.001 M gallic acid (Gurib-Fakim, 2006).

3.14 Mineral elements analysis

Mineral composition of the plant extracts was analyzed using total reflection X-ray fluorescence system (TRXF) and atomic absorption spectrometry (AAS).

3.14.1 Elemental analysis by total reflection x-ray fluorescence system (TXRF System)

TXRF system was used to determine the content of Sodium (Na), Magnesium (Mg), chlorine (Cl), potassium (K), Calcium (Ca), Titanium (Ti), Vanadium (V), manganese (Mn), iron (Fe), Copper (Cu), Zinc (Zn), Gallium (Ga), Arsenic (As), selenium (Se), Bromine (Br), Rubidium (Rb), Strontium (Sr), Nickel (Ni), Lead
(Pb), and Uranium (U) in the lyophilized plant samples as described by Hagen (2007).

3.14.1.1 Sample preparation

Samples were prepared as follows:

I. 1 g each (3 sets) of lyophilized sample were weighed into clean vials.

II. 10mL of double distilled water were added to each sample for dissolution.

III. 20μL of 1000ppm gallium stock solution was added into each sample (as internal standard) resulting into a concentration of 2ppm Ga in each sample.

IV. Each sample was homogenized for 1 minute using a vortex mixer.

V. Aliquots of 10μL of each sample were pipetted out using a micropipette onto a clean quartz carrier.

VI. The carriers were then dried in an oven to evaporate the liquid.

3.14.1.2 TXRF system

The main principle of X-ray Fluorescence Spectroscopy (XRF) is that when atoms are irradiated with X-rays, they emit secondary X-rays called fluorescence radiation. These fluorescence radiations are characteristic for a particular atom (element) and are of specific energy which makes it possible for qualitative and quantitative analyses.
3.14.1.3 Sample spectrum acquisition and quantitative analysis

I. Each sample carrier was irradiated for 1000 seconds using a S2 PICOFOX TXRF Spectrometer which was operated at 50kV and a current of 1000μA. The spectrometer uses a molybdenum anode.

II. Evaluation of the measured spectra was done using S2 PICOFOX software on the basis of the chosen elements.

III. Using the same software (S2 PICOFOX), concentrations were calculated based on the net intensities of the element peaks as per the following formula;

\[
C_{is} = \frac{N_x}{N_{is}} \times C_{is}
\]

Where,

\begin{align*}
C_x & \quad \text{Concentration of the analyte} \\
C_{is} & \quad \text{Concentration of the internal standard} \\
N_x & \quad \text{Net intensity of the analyte} \\
N_{is} & \quad \text{Net intensity of the internal standard} \\
S_x & \quad \text{Relative sensitivity of analyte} \\
S_{is} & \quad \text{Relative sensitivity of internal standard}
\end{align*}

3.14.2 Atomic absorption spectrophotometry (AAS)

This technique was used for the analysis of Magnesium, Chromium and Cadmium. Samples and standard solutions were prepared as described by Piero et
al (2012e) with slight modifications. Suitable amounts of standard stock solutions of each element were taken in a series of 100ml volumetric flasks.

The solutions were diluted to volume using distilled-deionized water, mixed thoroughly and transferred into plastic beakers. Working standard solutions for each element were prepared within a given range (1ppm, 5ppm, 10ppm, 15ppm, 20ppm, and 25ppm). Standard blank reagents for each element were prepared by adding all the reagents, except the target element being determined. No sample digestion was required as the freeze dried aqueous extracts of the plant samples were used.

After setting the AAS instrument to the right conditions for each element, the respective standard and sample solutions were aspirated into the flame in turns to determine their respective absorbance. Distilled-deionized water was always flushed into the flame to re-establish the zero absorbance. The procedure was repeated two times for each sample and element. The mean absorbance for each sample solution and standard solutions were calculated and recorded. Calibration curve for each element was prepared by plotting a graph of mean absorbance against corresponding concentrations of the standard solutions. Microsoft Excel computer software was used to convert absorbance readings to concentrations of elements in each sample analyzed with better accuracy than manual graphical method. The program gave concentrations of the diluted and undiluted samples
directly. Concentration values obtained for the diluted samples were corrected by multiplying with the respective dilution factors. The final values expressed as mg/g.

3.15 Data management and statistical analysis

The Data was entered in the Microsoft Excel Spread Sheet, cleaned and then exported to Statistical Package of Social Sciences (SPSS) Software for analysis. Results were expressed as Mean ± Standard Deviation (SD) of the number of animals used per every study point. Statistical analysis were done using ANOVA and post-ANOVA (Turkey) to compare the means of untreated normal control mice with diabetic mice treated with saline, diabetic mice treated with the conventional drug, and diabetic mice treated with plant extracts at doses of 50mg/kg body weight, 100mg/kg body weight, 200mg/kg body weight, and 300mg/kg body weight. For in vivo toxicity test unpaired student t-test was used to compare the data of normal control group with the group treated with the extract. p ≤ 0.05 was considered statistically significant.
CHAPTER FOUR

RESULTS

4.1 In vivo hypoglycemic assays

4.1.1 Effect of oral and intraperitoneal administration of aqueous stem bark extracts of *Kleinia squarrosa* on blood glucose levels in alloxan induced diabetic mice.

The dry powder of *Kleinia squarrosa* yielded 3.98% (w/w) aqueous stem bark extract. Treatment with the aqueous stem bark extracts showed hypoglycemic properties in alloxan induced diabetic mice (Table 4.1; Figure 4.1 and 4.2). Oral administration of the aqueous stem bark extracts of *Kleinia squarrosa* decreased the blood glucose levels at all the four tested dose levels (50, 100, 200 and 300 mg/kg body weight). As shown in Figure 1, during the 1st hour the percent reductions of the four therapeutic doses in the blood glucose levels were 41.32, 45.60, -0.92, and 14.17%, respectively, compared to the reference drug glibenclamide which lowered blood glucose levels by 9.51% within the same hour. In this hour the four tested dose levels did not lower blood glucose levels to normal. The aqueous stem bark extracts of *Kleinia squarrosa* decreased the blood glucose levels appreciably by the four therapeutic doses in the 4th and 6th hour.

In the 4th hour the percent blood glucose reductions were 53.63, 50.06, 46.15, and 59.46%, respectively, compared to glibenclamide which lowered blood glucose levels by 59.37% within the same hour. In this hour only dose level 300 mg/kg body weight decreased blood glucose levels to normal. In the 6th hour the percent
blood glucose reductions were 54.78, 49.50, 56.00 and 70.42%, respectively, compared to glibenclamide which lowered blood sugar levels by 76.18% within the same hour. In this hour only the dose levels 300 mg/kg body weight lowered blood glucose levels to normal and was effective as glibenclamide. After this, there was a gradual increase up to the twenty fourth hours (Table 4.1; Figure 4.1).

Intraperitoneal administration of aqueous stem bark extracts of the four therapeutic doses (50, 100, 200 and 300mg/kg body weight) of *Kleinia squarrosa* also lowered blood glucose levels from the 1st hour to the 6th hour (Table 4.1; Figure 4.2). By the 1st hour the four therapeutic doses of the extract had lowered the blood glucose levels by 42.24, 56.89, 20.49, and 27.17%, respectively, compared to insulin which had lowered blood sugar levels by 70.09% within the same hour. In this hour all tested dose levels did not lower blood glucose levels to normal. In the 6th hour the percent blood glucose reductions by the four therapeutic doses of the extract were 65.03, 72.83, 71.56 and 58.68%, respectively, compared to insulin which had lowered blood sugar levels by 73.52% within the same hour. In this hour the four tested dose levels lowered blood glucose levels to normal and were effective as insulin. After this, a gradual increase was recorded up to the twenty fourth hour.
Table 4.1: Effect of oral and intraperitoneal administration of aqueous stem bark extracts of *Kleinia squarrosa* on blood glucose levels in alloxan induced diabetic mice.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Route</th>
<th>Glucose levels at varying times in (mmol/L)</th>
<th>Extract dose (mg/kg body weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0hr</td>
<td>1hr</td>
</tr>
<tr>
<td>Control</td>
<td>Oral</td>
<td>5.18±0.25</td>
<td>5.00±0.21</td>
</tr>
<tr>
<td></td>
<td>IP</td>
<td>5.14±0.17</td>
<td>5.04±0.17</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>Oral</td>
<td>18.92±2.19^A</td>
<td>20.98±2.38^C</td>
</tr>
<tr>
<td></td>
<td>IP</td>
<td>19.90±1.02^A</td>
<td>22.22±1.74^Ba</td>
</tr>
<tr>
<td>Diabetic/Glib</td>
<td>Oral</td>
<td>20.82±2.17^Ad</td>
<td>18.84±2.29^Dd</td>
</tr>
<tr>
<td></td>
<td>IP</td>
<td>21.60±1.53^Bb</td>
<td>6.46±0.81</td>
</tr>
</tbody>
</table>

Values are expressed as Means ± SD for five animals per group. Means within respective columns followed by similar upper case letters are not significantly different at *p* ≤ 0.05 by ANOVA and post ANOVA; means within respective rows followed by similar lower case letters are not significantly different at *p* ≤ 0.05 by ANOVA and post ANOVA; and post ANOVA.
Figure 4.1: The mean percentage change in blood glucose levels after aqueous stem bark extract of *Kleinia squarrosa* was administered orally in alloxan induced diabetic mice.
Figure 4.2: The mean percentage change in blood glucose levels after aqueous stem bark extract of *Kleinia squarrosa* was administered intraperitoneally in alloxan induced diabetic mice.
4.1.2 Effect of oral and intraperitoneal administration of aqueous stem bark extracts of *Acacia nilotica* on blood glucose levels in alloxan induced diabetic mice.

The dry powder of *Acacia nilotica* yielded 5.63% (w/w) aqueous stem bark extracts. At the four therapeutic dose levels (50, 100, 200 and 300mg/kg body weight) oral administration of the aqueous stem bark extracts of *Acacia nilotica* decreased the blood glucose levels (Table 4.2; Figure 4.3). During the 1st hour the percent reductions in the blood glucose levels were 38.72, 25.84, 28.83, and 17.15%, respectively, compared to reference drug glibenclamide which lowered blood glucose levels by 12.52% within the same hour. In this hour the four tested dose levels did not lower blood glucose levels to normal. By the third hour, all the four therapeutic dose levels (50, 100, 200 and 300mg/kg body weight) lowered blood glucose levels by more than half, that is, 69.01, 58.32, 52.44 and 50.67%, respectively, compared to glibenclamide which lowered blood glucose levels by 46.97% within the same hour. However none of the four tested dose levels decreased blood glucose levels to normal in the third hour.

In the 6th hour the percent blood glucose reductions by the four therapeutic doses were 81.92, 77.43, 63.13 and 64.27%, respectively, compared to glibenclamide which lowered blood glucose levels by 75.88% within the same hour. In this hour all tested dose levels decreased blood glucose levels to normal and were effective as glibenclamide. After this, a gradual increase was recorded up to the twenty fourth hour.
Intraperitoneal administration of aqueous stem bark extracts at all the four therapeutic doses of *Acacia nilotica* also lowered blood glucose levels from the 1st hour to the 6th hour (Table 4.2; Figure 4.4) in a dose independent manner. By the 1st hour the four therapeutic doses of the extract had lowered the blood glucose levels by 49.55, 41.60, 26.26, and 50.32% respectively, compared to insulin which had lowered blood sugar levels by 65.04% within the same hour. In this hour the dose levels 50mg/kg body weight and 300mg/kg body weight lowered blood glucose levels to normal and were effective as insulin. However the dose levels 100mg/kg body weight and 200mg/kg body did not lowered blood glucose levels to normal.

In the 6th hour the percent blood glucose reductions by the four therapeutic doses were 60.96, 53.66, 60.88 and 62.10%, respectively, compared to insulin which had lowered blood sugar levels by 77.59% within the same hour. In this hour all tested dose levels lowered blood glucose levels to normal and were effective as insulin. After this, a gradual increase was recorded up to the twenty fourth hour.
Table 4.2: Effect of oral and intraperitoneal administration of aqueous stem bark extracts of *Acacia nilotica* on blood glucose levels in alloxan induced diabetic mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Route</th>
<th>Glucose levels at varying times in mmol/dL</th>
<th>0hr</th>
<th>1hr</th>
<th>2hr</th>
<th>3hr</th>
<th>4hr</th>
<th>6hr</th>
<th>24hr</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>Oral</td>
<td>5.30±0.28</td>
<td>5.04±0.17</td>
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<td>5.12±0.23</td>
<td>5.08±0.08</td>
<td>5.18±0.25</td>
<td>4.96±0.21</td>
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</tr>
<tr>
<td></td>
<td>IP</td>
<td>5.12±0.18</td>
<td>5.28±0.22</td>
<td>5.28±0.13</td>
<td>5.12±0.22</td>
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<td>Diabetic control</td>
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<tr>
<td></td>
<td>IP</td>
<td>19.84±1.26A</td>
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<td>23.08±1.50B</td>
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<td>Diabetic/Glib</td>
<td>Oral</td>
<td>23.80±2.72Ac</td>
<td>20.82±2.43Ab</td>
<td>17.62±3.15Ab</td>
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<td>7.30±0.54Ab</td>
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<td>Extract dose (mg/kg body weight)</td>
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<td>25.88±1.12Ac</td>
<td>15.86±2.50Ab</td>
<td>11.78±5.33Ab</td>
<td>8.02±3.14</td>
<td>5.18±1.62</td>
<td>4.68±1.33</td>
<td>6.66±1.09</td>
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<tr>
<td></td>
<td>IP</td>
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<td>8.22±2.07</td>
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<tr>
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<td>9.42±0.89Ab</td>
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<td>24.14±4.12Ac</td>
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<td>5.50±0.50</td>
<td>5.92±0.71</td>
<td>7.48±1.58</td>
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</table>

Values are expressed as Means ± SD for five animals per group. Means within respective columns followed by similar upper case letters are not significantly different at p ≤ 0.05 by ANOVA and post ANOVA; means within respective rows followed by similar lower case letters are not significantly different at p ≤ 0.05 by ANOVA and post ANOVA.
Figure 4.3: The mean percentage change in blood glucose levels after aqueous stem bark extract of *Acacia nilotica* was administered orally in alloxan induced diabetic mice.
Figure 4.4: The mean percentage change in blood glucose levels after aqueous stem bark extract of *Acacia nilotica* was administered intraperitoneally in alloxan induced diabetic mice.
4.1.3 Effect of oral and intraperitoneal administration of aqueous leaf extracts of *Zanha africana* on blood glucose levels in alloxan induced diabetic mice.

The dry powder of *Zanha africana* yielded 3.45% (w/w) aqueous leaf extract. Oral administration of aqueous leaf extracts of *Zanha africana* at the four therapeutic dose levels (50, 100, 200 and 300mg/kg body weight) decreased the blood glucose levels from the 1<sup>st</sup> hour to the 6<sup>th</sup> hour in a dose independent manner. Thereafter, there was a gradual increase up to the 24<sup>th</sup> hour (Table 4.3; Figure 4.5). During the 1<sup>st</sup> hour the percent reductions in the blood glucose levels by the four aqueous leaf extract doses were 37.87, 10.38, 14.76, and 14.06%, respectively, compared to reference drug glibenclamide which lowered blood glucose levels by 8.87% within the same hour. In this hour none of the four tested dose levels lowered blood glucose levels to normal.

In the 6<sup>th</sup> hour the percent blood glucose reductions by the four aqueous leaf extract doses were 64.89, 29.77, 59.78 and 58.19% respectively, compared to glibenclamide which lowered blood glucose levels by 77.13% within the same hour. In this hour only dose level 50mg/kg body weight lowered blood glucose levels to normal and was effective as glibenclamide. After this, a gradual increase was recorded up to the twenty fourth hour.

Intraperitoneal administration of aqueous leaf extract at all four dose levels (50, 100, 200 and 300mg/kg body weight) of *Zanha africana* also lowered blood
glucose levels from the 1st hour to the 6th hour (Table 4.3; Figure 4.6) in a dose independent manner. By the 1st hour the four extract doses had lowered the blood glucose levels by 6.91, 10.70, 13.25, and 13.70%, respectively, compared to insulin which had lowered blood sugar levels by 68.40% within the same hour. In this hour all tested dose levels did not lower blood glucose levels to normal.

By the 6th hour, all the four dose levels (50, 100, 200 and 300mg/kg body weight) of *Zanha africana* lowered blood glucose levels by more than half, that is, 70.22, 55.49, 57.65 and 63.56%, respectively, compared to insulin which had lowered blood glucose levels by 76.30% within the same hour. In this hour dose level 50 mg/kg body weight lowered blood glucose levels to normal and was effective as insulin while the dose 200mg/kg body weight also lowered blood glucose levels to normal but was not effective as insulin. However, dose levels 100mg/kg body weight and 300mg/kg body did not lower blood glucose levels to normal in this hour. After this, a gradual increase was recorded up to the twenty fourth hour.
Table 4.3: Effect of oral and intraperitoneal administration of aqueous leaf extracts of *Zanthoxylum africana* on blood glucose levels in alloxan induced diabetic mice.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Route</th>
<th>Glucose levels at varying times in mmol/L:</th>
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<td>6hr</td>
<td>24hr</td>
</tr>
<tr>
<td>Normal control</td>
<td>Oral</td>
<td>5.12±0.15</td>
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<td>5.18±0.13</td>
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<td>Diabetic control</td>
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<td>25.34±1.80Ba</td>
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</tr>
<tr>
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<tr>
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<td>Oral</td>
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<td>19.32±7.27Aa</td>
<td>16.50±7.16A</td>
<td>14.86±5.76A</td>
<td>11.62±4.13A</td>
<td>9.40±1.74A</td>
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<td>IP</td>
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<td>15.12±4.66B</td>
<td>12.84±3.60B</td>
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</tbody>
</table>

Values are expressed as Means ± SD for five animals per group. Means within respective columns followed by similar upper case letters are not significantly different at p ≤ 0.05 by ANOVA and post ANOVA; means within respective rows followed by similar lower case letters are not significantly different at p ≤ 0.05 by ANOVA and post ANOVA.
Figure 4.5: The mean percentage change in blood glucose levels after aqueous leaf extracts of *Zanha africana* was administered orally in alloxan induced diabetic mice.
Figure 4.6: The mean percentage change in blood glucose levels after aqueous leaf extracts of *Zanha africana* was administered intraperitoneally in alloxan induced diabetic mice.
4.1.4 Effect of oral and intraperitoneal administration of aqueous stem bark extracts of *Aloe secundiflora* on blood glucose levels in alloxan induced diabetic mice.

The dry powder of *Aloe secundiflora* yielded 7.55% (w/w) aqueous stem bark extract. Table 4.4, figure 4.7 and 4.8 show the pattern of blood glucose reduction by four aqueous stem bark extract doses (50, 100, 200 and 300mg/kg body weight) of *Aloe secundiflora*. In the 1st hour, oral administration of the four aqueous stem bark extract doses (50, 100, 200 and 300mg/kg body weight) caused a percent reduction in the blood glucose levels of 16.48, 6.71, 15.19, and 7.69%, respectively, compared to the reference drug glibenclamide which lowered blood glucose levels by 8.28% within the same hour.

As table 4 shows at this hour the aqueous stem bark extract dose levels of 100, 200, and 300mg/kg body weight insignificantly lowered blood glucose levels. However the aqueous extract dose level of 50mg/kg body weight significantly lowered the blood glucose levels in the 1st hour. In the 6th hour the percent blood glucose reductions by the four aqueous extract doses were 63.83, 10.89, 36.60 and 9.11%, respectively, compared to glibenclamide which lowered blood glucose levels by 78.56% within the same hour. At this hour only the dose level 50mg/kg body weight significantly lowered blood glucose levels to normal and was effective as glibenclamide, while the other doses insignificantly lowered the blood glucose levels at this hour. After this, a gradual increase was recorded up to the twenty fourth hour.
As shown in table 4.4 and figure 4.8 after intraperitoneal administration of stem bark extract of *Aloe secundiflora* only the dose level 300mg/kg body weight had shown significant and consistence reduction in the blood glucose levels. The percent blood glucose reductions of this aqueous stem bark extract dose were 60.96% in the 1\textsuperscript{st} hour and 68.57% in the 6\textsuperscript{th} hour. After this, a gradual increase was recorded in the 24\textsuperscript{th} hours. As shown in table 4.4 this dose level lowered blood glucose levels to normal in the 6\textsuperscript{th} hour.

The aqueous stem bark extract dose level of 50mg/kg body weight significantly lowered blood glucose levels by 15.50% in the 1\textsuperscript{st} hour. After this, a gradual increase was recorded from 2\textsuperscript{nd} to 6\textsuperscript{th} hour. In the 24\textsuperscript{th} the dose again significantly lowered blood glucose levels by 60% but not to normal. The aqueous stem bark extract dose level at 100mg/kg body weight did not significantly lower the blood glucose levels from 1\textsuperscript{st} to 6\textsuperscript{th} hour. However the dose insignificantly lowered blood glucose levels by 27.26% in the 24\textsuperscript{th} hour. The aqueous stem bark extract dose level of 200mg/kg body weight had only shown insignificant reduction in blood glucose levels by 12.59% and 12.18% in the 6\textsuperscript{th} and 24\textsuperscript{th} hour.
Table 4.4: Effect of oral and intraperitoneal administration of aqueous stem bark extracts of *Aloe secundiflora* on blood glucose levels in alloxan induced diabetic mice.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Route</th>
<th>Glucose levels at varying times in mmol/L</th>
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<th>1hr</th>
<th>2hr</th>
<th>3hr</th>
<th>4hr</th>
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</tr>
</thead>
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<tr>
<td>Normal control</td>
<td>Oral</td>
<td>5.22±0.43</td>
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<td>Extract dose (mg/kg body weight)</td>
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<tr>
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<td>11.34±5.31A</td>
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<td>6.54±1.56A</td>
<td>9.16±4.31A</td>
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<tr>
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<td>25.16±2.63Aa</td>
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Figure 4.7: The mean percentage change in blood glucose levels after aqueous stem bark extract of *Aloe secundiflora* was administered orally in alloxan induced diabetic mice.
Figure 4.8: The mean percentage change in blood glucose levels after aqueous stem bark extract of *Aloe secundiflora* was administered intraperitoneally in alloxan induced diabetic mice.
Figure 4.8: The mean percentage change in blood glucose levels after aqueous stem bark extract of *Aloe secundiflora* was administered intraperitoneally in alloxan induced diabetic mice.
4.1.5 Effect of oral and intraperitoneal administration of aqueous stem bark extracts of *Fuerstia africana* on blood glucose levels in alloxan induced diabetic mice.

The dry powder of *Fuerstia africana* yielded 3.58% (w/w) aqueous stem bark extract. As table 4.5 and figure 4.9 shows, oral administration of aqueous stem bark extract of the four dose levels (50, 100, 200 and 300mg/kg body weight) did not significantly lower blood glucose levels during the whole experimental period.

After intraperitoneal administration of the aqueous stem bark extract dose levels (50, 100, 200 and 300mg/kg body weight) of *Fuerstia africana*, only the dose level of 300mg/kg body weight demonstrated slight but significant (p ≤0.05) reduction in blood glucose levels from the 1st hour to the 6th hour (Table 4.5; Figure 4.10). The percent reductions in the blood glucose levels of the aqueous stem bark extract at this dose level was 5.94% in the 1st hour and 25.47% in the 6th hour. However this dose levels did not lower blood glucose levels to normal during the experimental period.
Table 4.5: Effect of oral and intraperitoneal administration of aqueous stem bark extracts of *Fuerstia africana* on blood glucose levels in alloxan induced diabetic mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Route</th>
<th>Glucose levels at varying times in mmol/L</th>
<th>0hr</th>
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Extract dose (mg/kg body weight)

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<td>IP</td>
<td>12.80±1.91A</td>
<td>12.04±1.40A</td>
<td>11.00±0.96A</td>
<td>9.90±1.34A</td>
<td>11.48±1.70A</td>
<td>9.54±1.42</td>
<td>12.10±1.11A</td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed as Means ± SD for five animals per group. Means within respective columns followed by similar upper case letters are not significantly different at p ≤0.05 by ANOVA and post ANOVA; means within respective rows followed by similar lower case letters are not significantly different at p ≤0.05 by ANOVA and post ANOVA.
Figure 4.9: The mean percentage change in blood glucose levels after aqueous stem bark extract of *Fuerstia africana* was administered orally in alloxan induced diabetic mice.
Figure 4.10: The mean percentage change in blood glucose levels after aqueous stem bark extract of *Fuerstia africana* was administered intraperitoneally in alloxan induced diabetic mice.
4.2 *In vivo* single toxicity studies

4.2.1 Effect of oral and intraperitoneal administration of aqueous plants extracts at 1g/kg body weight on body and organ weights in mice.

The effect of oral and intraperitoneal administration of different aqueous plant extracts at 1g/kg body weight to mice for one month on the weekly change in body weight and percent organ to body weight is shown in tables 4.6, 4.7, 4.8 and 4.9. Oral administration 1g/kg body weight of aqueous stem bark extracts of *Acacia nilotica*, *Fuerstia africana*, and *Aloe secundiflora* and leaf extracts of *Zanha africana* to mice for one month did not significantly alter the percent organ to body weights of all the studied organs relative to those of the normal control mice (Table 4.6). However, oral administration of aqueous leaf extracts of *Zanha africana*, and aqueous stem bark extracts of *Kleinia squarrosa* at 1g/kg body weight to mice for one month significantly decreased the weekly body weight gain relative to that of the normal control mice (Table 4.8). Intraperitoneal administration of aqueous stem bark extracts of *Acacia nilotica*, *Fuerstia africana*, *Aloe secundiflora* and *Kleinia squarrosa*, and leaf extracts of *Zanha africana* to mice for one month significantly decreased the weekly body weight gain relative to that of the normal control mice (Table 4.9).

Intraperitoneal administration of the aqueous stem bark extracts of *Acacia nilotica* at 1g/kg body weight to mice for one month significantly decreased the percent organ to body weight of testes while not significantly altering the percent organ to
body weight of the other studied organs relative to those of the normal control mice (Table 4.9). In addition, administration of the same intraperitoneal dose of aqueous leaf extracts of *Zanha africana* to mice for one month significantly increased the percent organ to body weight of liver, brain, and kidney but did not significantly alter the percent organ to body weight of the lungs, spleen, heart, and testes relative to those of the normal control mice (Table 4.9).

Intraperitoneal administration of aqueous stem bark extracts of *Fuerstia africana* at 1g/kg body weight to mice for one month significantly increased the percent organ to body weight of the brain but did not significantly alter the percent organ to body weight of the liver, kidney, lungs, spleen, heart, and testes relative to those of the normal control mice (Table 4.9). Further, administration of the same intraperitoneal dose of aqueous stem bark extracts of *Aloe secundiflora* to mice for one month significantly increased the percent organ to body weight of brain while not significantly altering the percent organ to body weight of the liver, kidney, lungs, spleen, heart, and testes relative to those of the normal control mice (Table 4.9).

Oral administration of 1g/kg body weight of aqueous stem bark extracts of *Kleinia squarrosa* to mice for one month significantly decreased the percent organ to body weight of liver but did not significantly alter the percent organ to body weight of the brain, kidney, lungs, spleen, heart and testes relative to those
of the normal control mice (Table 4.8). In addition, administration of the same intraperitoneal dose of aqueous stem bark extracts of *Kleinia squarrosa* to mice significantly increased the percent organ to body weight of liver and lungs while not altering the percent organ to body weight of the brain, kidney, spleen, heart, and testes relative to those of the normal control mice (Table 4.9).
Table 4.6: The effects of oral administration of 1g/kg body weight of aqueous plant extracts on average weekly body weight and weekly change in body weight in mice.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Average weekly mice weight (g)</th>
<th>Average weekly change in body weight (g/week)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>14.0±1.4</td>
<td>1.705±0.522</td>
</tr>
<tr>
<td>Acacia nilotica</td>
<td>13.8±0.4</td>
<td>0.940±0.554</td>
</tr>
<tr>
<td>Zanthoxylum piperitum</td>
<td>18.6±3.1*</td>
<td>0.340±0.495*</td>
</tr>
<tr>
<td>Fuerstia africana</td>
<td>14.2±1.9</td>
<td>1.730±0.808</td>
</tr>
<tr>
<td>Aloe secundiflora</td>
<td>17.2±4.4</td>
<td>1.235±0.775</td>
</tr>
<tr>
<td>Kleinia squarrosa</td>
<td>17.8±3.3*</td>
<td>-0.125±0.265*</td>
</tr>
</tbody>
</table>

Average weekly mice weight and average weekly change in mice body weight are measured in g. Results are expressed as Mean ± Standard Deviation (SD) for five animals for each parameter; *p < 0.05 is considered statistically significant when the mean of the experiment group for each plant extract is compared to its relevant control group by t-test.
Table 4.7: The effects of intraperitoneal administration of aqueous plant extracts at 1g/kg body weight on average weekly body weight and weekly change in body weight in mice.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Average weekly mice weight (g)</th>
<th></th>
<th></th>
<th></th>
<th>Weight/week</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>15.3±2.5</td>
<td>16.7±3.1</td>
<td>18.9±3.3</td>
<td>21.5±2.8</td>
<td>24.0±2.4</td>
</tr>
<tr>
<td>Acacia nilotica</td>
<td>21.2±2.4*</td>
<td>22.0±1.9*</td>
<td>23.0±1.7*</td>
<td>23.6±1.6</td>
<td>24.5±1.7</td>
</tr>
<tr>
<td>Zanha africana</td>
<td>13.0±1.2</td>
<td>13.4±1.1</td>
<td>13.7±1.0*</td>
<td>14.2±1.1*</td>
<td>15.0±0.8*</td>
</tr>
<tr>
<td>Fuerstia africana</td>
<td>17.4±0.8</td>
<td>17.3±0.8</td>
<td>17.3±0.8</td>
<td>18.2±0.8*</td>
<td>19.0±0.9*</td>
</tr>
<tr>
<td>Aloe secundiflora</td>
<td>13.7±1.1</td>
<td>15.1±1.0</td>
<td>15.6±1.0</td>
<td>16.8±1.1*</td>
<td>18.2±1.2*</td>
</tr>
<tr>
<td>Kleintia squarrosa</td>
<td>17.0±1.4</td>
<td>17.1±1.5</td>
<td>17.1±1.5</td>
<td>D</td>
<td>D</td>
</tr>
</tbody>
</table>

Average weekly mice weight and average weekly change in mice body weight are measured in g. Results are expressed as Mean ± Standard Deviation (SD) for five animals for each parameter; *p < 0.05 is considered statistically significant when the mean of the experiment group for each plant extract is compared to its relevant control group by t-test. D indicates that all the five mice died in the third week after the start of the experiment.
Table 4.8: The effects of oral administration of aqueous plant extracts at 1g/kg body weight on the percent organ to body weight in mice.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Percent organ to body weight (g)</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver</td>
<td>Brain</td>
<td>Kidney</td>
<td>Lungs</td>
<td>Spleen</td>
<td>Heart</td>
<td>Testes</td>
</tr>
<tr>
<td>Control</td>
<td>7.27±1.12</td>
<td>2.74±0.27</td>
<td>1.78±0.24</td>
<td>1.79±0.21</td>
<td>1.12±0.32</td>
<td>0.53±0.09</td>
<td>0.93±0.16</td>
</tr>
<tr>
<td><em>Acacia nilotica</em></td>
<td>7.93±1.11</td>
<td>3.30±0.61</td>
<td>1.89±0.18</td>
<td>1.85±0.44</td>
<td>1.14±0.21</td>
<td>0.56±0.15</td>
<td>0.77±0.22</td>
</tr>
<tr>
<td><em>Zanha africana</em></td>
<td>7.83±1.36</td>
<td>2.65±0.92</td>
<td>1.87±0.11</td>
<td>2.28±0.92</td>
<td>1.05±0.41</td>
<td>0.62±0.08</td>
<td>0.92±0.12</td>
</tr>
<tr>
<td><em>Fuerstia africana</em></td>
<td>8.07±2.42</td>
<td>2.93±0.43</td>
<td>1.90±0.52</td>
<td>1.77±0.32</td>
<td>1.14±0.25</td>
<td>0.58±0.24</td>
<td>1.07±0.18</td>
</tr>
<tr>
<td><em>Aloe secundiflora</em></td>
<td>7.73±1.25</td>
<td>2.85±0.52</td>
<td>1.78±0.23</td>
<td>2.03±0.48</td>
<td>1.35±0.28</td>
<td>0.58±0.04</td>
<td>0.82±0.18</td>
</tr>
<tr>
<td><em>Kleinia squarrosa</em></td>
<td>5.18±1.49*</td>
<td>3.22±0.52</td>
<td>1.88±0.22</td>
<td>1.92±0.36</td>
<td>1.28±0.29</td>
<td>0.57±0.11</td>
<td>0.98±0.22</td>
</tr>
</tbody>
</table>

Results are expressed as Mean ± Standard Deviation (SD) for five animals for each parameter; *p < 0.05 is considered statistically significant when the mean of the experiment group for each plant extract is compared to its relevant control group by t-test.
Table 4.9: The effects of intraperitoneal administration of 1g/kg body weight of aqueous plant extracts on percent organ to body weight in mice.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Percent organ to body weights (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver</td>
</tr>
<tr>
<td>Control</td>
<td></td>
</tr>
<tr>
<td>Acacia nilotica</td>
<td>5.01±1.26</td>
</tr>
<tr>
<td>Zanthoxylon aficdiana</td>
<td>4.75±0.48</td>
</tr>
<tr>
<td>Fuerstia africana</td>
<td>9.18±1.74*</td>
</tr>
<tr>
<td>Aloe secundiflora</td>
<td>6.31±1.48</td>
</tr>
<tr>
<td>Kleinia squarrosa</td>
<td>7.75±1.37*</td>
</tr>
</tbody>
</table>

Results are expressed as Mean ± Standard Deviation (SD) for five animals for each parameter; *p < 0.05 is considered statistically significant when the mean of the experiment group for each plant extract is compared to its relevant control group by t-test.
4.2.2 Effect of oral and intraperitoneal administration of 1g/kg body weight of different plant extracts on hematological parameters in mice.

The effect of oral and intraperitoneal administration of different plant extracts for one month on some hematological parameters in mice is shown in tables 4.10 and 4.11. Oral administration of 1g/kg body weight of aqueous stem bark extracts of *Acacia nilotica* to mice for one month significantly decreased the level of PLT but did not significantly change the levels of RBC, Hb, PCV, MCV, MCH, and MCHC relative to those of the normal control mice. In addition, administration of the same intraperitoneal dose of aqueous stem bark extracts of *Acacia nilotica* and aqueous leaf extracts of *Zanha africana*, and oral dose of aqueous stem bark extracts of *Fuerstia africana* to mice for one month did not significantly change the levels of all the measured hematological parameters relative to those of the normal control mice.

Oral administration of 1g/kg body weight of aqueous leaf extracts of *Zanha africana* to mice for one month significantly increased the level of MCH and significantly decreased the level of PLT but did not significantly change the levels of RBC, Hb, PCV, MCV, and MCHC relative to those of the normal control mice (Table 4.10). In addition, administration of the same intraperitoneal dose of aqueous stem bark extracts of *Fuerstia africana* to mice for one month significantly decreased the levels of RBC and PCV but did not significantly
change the levels of Hb, MCV, MCH, MCHC and PLT relative to those of the normal control mice (Table 4.11).

Oral administration of 1g/kg body-weight of *Aloe secundiflora* aqueous stem bark extracts to mice for one month significantly decreased the level of PLT, but did not significantly change the levels of RBC, Hb, PCV, MCV, MCH, and MCHC relative to those of the normal control mice (Table 4.10). In addition, administration of the same intraperitoneal dose of aqueous stem bark extracts of *Aloe secundiflora* to mice for one month significantly increased the level of MCHC but did not significantly change the levels of RBC, HGB, PCV, MCV, MCH, and PLT relative to those of the normal control mice (Table 4.11).

Oral administration of 1g/kg body weight of *Kleinia squarrosa* aqueous stem bark extracts to mice for one month significantly decreased the level of RBC but did not significantly change the levels of Hb, PCV, MCV, MCH, MCHC, and PLT relative to those of the normal control mice (Table 4.10). In addition, administration of the same intraperitoneal dose of *Kleinia squarrosa* aqueous stem bark extract to mice significantly decreased the levels of RBC, Hb, PCV, and PLT and increased the levels of MCH and MCHC but did not significantly affect the level of MCV relative to those of the normal control mice (Table 4.11).
Table 4.1: The effects of oral administration of Ig/kg body weight of aqueous plant extracts on hematological parameters in mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>RBC (x10⁶/µL)</th>
<th>Hb (g/dL)</th>
<th>PCV (%)</th>
<th>MCV (fl)</th>
<th>MCH (pg)</th>
<th>MCHC (g/dL)</th>
<th>PLT (x10⁹/µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7.61±0.74</td>
<td>9.58±0.73</td>
<td>32.74±2.49</td>
<td>43.12±2.84</td>
<td>12.64±0.96</td>
<td>29.30±0.54</td>
<td>607.40±116.07</td>
</tr>
<tr>
<td><em>Acacia nilotica</em></td>
<td>8.45±1.32</td>
<td>10.24±1.57</td>
<td>36.00±5.68</td>
<td>42.60±2.08</td>
<td>12.42±0.93</td>
<td>29.12±1.06</td>
<td>422.68±69.92</td>
</tr>
<tr>
<td><em>Zantha africana</em></td>
<td>6.69±1.19</td>
<td>10.08±1.05</td>
<td>30.96±5.48</td>
<td>46.26±2.41</td>
<td>14.40±1.06</td>
<td>31.16±3.21</td>
<td>436.32±57.54</td>
</tr>
<tr>
<td><em>Fuerstia africana</em></td>
<td>8.49±1.18</td>
<td>10.60±0.82</td>
<td>36.42±3.24</td>
<td>43.24±3.67</td>
<td>12.66±1.74</td>
<td>29.08±1.57</td>
<td>599.66±88.47</td>
</tr>
<tr>
<td><em>Aloe secundiflora</em></td>
<td>6.07±2.89</td>
<td>8.08±3.83</td>
<td>27.34±12.37</td>
<td>45.72±2.29</td>
<td>13.26±0.81</td>
<td>29.04±2.03</td>
<td>418.98±77.67</td>
</tr>
<tr>
<td><em>Kleina squarrosa</em></td>
<td>6.32±0.88*</td>
<td>8.04±1.38</td>
<td>28.22±4.30</td>
<td>44.14±1.38</td>
<td>12.66±0.42</td>
<td>28.62±0.58</td>
<td>482.40±43.62</td>
</tr>
</tbody>
</table>

Results are expressed as Mean ± standard deviation (SD) for five animals in each treatment; *p < 0.05 is considered significant when the mean of the control animals is significantly different from that of the extract treated animals by t-test.
Table 4.11: The effects of intraperitoneal administration of aqueous plant extracts at 1g/kg body weight on hematological parameters in mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Hematological parameters</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RBC (x10^6/μL)</td>
<td>Hb</td>
<td>PCV (%)</td>
<td>MCV (fL)</td>
<td>MCH (pg)</td>
<td>MCHC (g/dL)</td>
<td>PLT (X10^3/μL)</td>
</tr>
<tr>
<td>Control</td>
<td>6.38±0.67</td>
<td>8.98±0.80</td>
<td>28.66±2.64</td>
<td>45.02±1.44</td>
<td>14.10±0.51</td>
<td>31.36±0.66</td>
<td>352.80±73.32</td>
</tr>
<tr>
<td>Acacia nilotica</td>
<td>5.32±1.27</td>
<td>7.50±1.88</td>
<td>24.98±4.97</td>
<td>47.52±3.44</td>
<td>14.06±0.48</td>
<td>29.74±2.62</td>
<td>341.00±39.64</td>
</tr>
<tr>
<td>Zanzia africana</td>
<td>6.20±0.66</td>
<td>8.80±1.10</td>
<td>28.14±3.25</td>
<td>45.36±1.93</td>
<td>14.20±0.72</td>
<td>31.26±0.61</td>
<td>439.00±96.11</td>
</tr>
<tr>
<td>Fuerstia africana</td>
<td>5.17±0.89*</td>
<td>7.58±1.09</td>
<td>24.04±3.14*</td>
<td>47.10±3.74</td>
<td>14.78±1.08</td>
<td>31.38±0.70</td>
<td>325.80±46.45</td>
</tr>
<tr>
<td>Aloe secundiflora</td>
<td>5.66±1.03</td>
<td>8.04±1.27</td>
<td>25.06±3.45</td>
<td>44.96±2.88</td>
<td>14.62±0.67</td>
<td>32.60±0.95*</td>
<td>354.80±58.28</td>
</tr>
<tr>
<td>Kleinia squarrosa</td>
<td>1.91±0.15*</td>
<td>3.64±0.11*</td>
<td>8.96±0.35*</td>
<td>46.50±0.96</td>
<td>18.88±0.34*</td>
<td>40.42±1.28*</td>
<td>22.20±0.88*</td>
</tr>
</tbody>
</table>

Results are expressed as Mean ± standard deviation (SD) for five animals in each treatment; *p < 0.05 is considered significant when the mean of the control animals is significantly different from that of the extract treated animals by t-test.
4.2.3 Effect of oral and intraperitoneal administration of different aqueous plant extracts at 1g/kg body weight on differential white blood cell count in mice.

The effect of oral and intraperitoneal administration of different aqueous plant extracts at 1g/kg body weight to mice for one month on differential white blood cell count is shown in tables 4.12 and 4.13. Oral and intraperitoneal administration of aqueous stem bark extracts of *Acacia nilotica*, *Fuerstia africana*, and *Aloe secundiflora* at 1g/kg body weight to mice for one month did not cause significant change to the differential white blood cell count. In addition, administration of the same oral dose of aqueous leaf extracts of *Zanha africana*, and aqueous stem bark extracts of *Kleinia squarrosa* to mice for one month did not significantly change the differential white blood cell counts (Table 4.12).

Intraperitoneal administration of aqueous leaf extracts of *Zanha africana* at 1g/kg body weight to mice for one month significantly increased the levels of WBC and lymphocytes without significantly affecting the levels of neutrophils, eosinophils, monocytes, and basophils relative to those of normal control mice. In addition, administration of the same intraperitoneal dose of stem bark extract of *Kleinia squarrosa* to mice caused a significant decrease in the levels of WBC, neutrophils, lymphocytes, eosinophils, and monocytes without significantly altering the level of basophils relative to those of the normal control mice (Table 4.13).
Table 4.12: The effects of oral administration of Ig/kg body weight of aqueous plant extracts on differential white blood cell count (WBC) in mice.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>White blood cell and differential white blood cell count</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WBC (x10⁵/µL)</td>
</tr>
<tr>
<td>Control</td>
<td>14.34±3.48</td>
</tr>
<tr>
<td><em>Acacia nilotica</em></td>
<td>13.53±5.98</td>
</tr>
<tr>
<td><em>Zanha Africana</em></td>
<td>12.82±6.38</td>
</tr>
<tr>
<td><em>Fuerstia Africana</em></td>
<td>14.10±3.82</td>
</tr>
<tr>
<td><em>Aloe secundiflora</em></td>
<td>9.10±8.24</td>
</tr>
<tr>
<td><em>Kleinia squarrosa</em></td>
<td>11.76±1.93</td>
</tr>
</tbody>
</table>

Results are expressed as Mean ± Standard Deviation (SD) for five animals in each treatment; *p < 0.05 is considered significant when the mean of the control animals is significantly different from that of the extract treated animals by t-test.
Table 4.13: The effects of intraperitoneal administration of 1g/kg body weight of aqueous plant extracts on differential white blood cell count (WBC) in mice.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>White blood cell and differential white blood cell count</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WBC (x10³/μL)</td>
</tr>
<tr>
<td>Control</td>
<td>6.87±0.71</td>
</tr>
<tr>
<td>Acacia nilotica</td>
<td>6.74±2.19</td>
</tr>
<tr>
<td>Zanha africana</td>
<td>9.08±0.94*</td>
</tr>
<tr>
<td>Fuerstia africana</td>
<td>7.60±1.42</td>
</tr>
<tr>
<td>Aloe secundiflora</td>
<td>7.08±1.47</td>
</tr>
<tr>
<td>Kleinia squarrosa</td>
<td>3.58±0.56*</td>
</tr>
</tbody>
</table>

Results are expressed as Mean ± Standard Deviation (SD) for five animals in each treatment; *p < 0.05 is considered significant when the mean of the control animals is significantly different from that of the extract treated animals by t-test.
4.2.4 Effects of oral and intraperitoneal administration of 1g/kg body weight of different plant extracts on biochemical parameters in mice.

Oral administration of 1g/kg body weight of aqueous stem bark extracts of *Acacia nilotica* caused a significant increase in the levels of GGT, CK, and T-BIL while decreasing the levels of ALT, AST, AMY, and ALP relative to that of the normal control mice; however, there was no significant alteration in the levels of Urea, LDH, D-BIL, and Creatinine by the same extract dose compared to that of the respective control group (Tables 4.14 and 4.15). Intraperitoneal administration of the same dose of aqueous stem bark extracts of *Acacia nilotica* significantly decreased the level of AST while not altering the levels of Urea, ALT, GGT, CK, LDH AMY, ALP, T-BIL, D-BIL, and Creatinine compared to that of the respective normal control group (Tables 4.16 and 4.17).

Oral administration of 1g/kg body weight of aqueous leaf extracts of *Zanha africana* caused a significant increase in levels of GGT, LDH, and CK while significantly decreasing the levels of Urea, ALT, AST, T-BIL, and Creatinine relative to that of the normal control mice; however, no significant alteration in the levels of ALP, AMY, and D-BIL by the same extract dose compared to that of the respective normal control group (Tables 4.14 and 4.15). Intraperitoneal administration of the same dose of aqueous leaf extracts of *Zanha africana* significantly increased the levels of GGT, T-BIL and D-BIL while decreasing the levels of AST and creatinine relative to that of the normal control mice; however,
no significant alteration on the levels of Urea, ALT, LDH, CK, ALP, and AMY by the same extract dose compared to respective normal control group (Tables 4.16 and 4.17).

Oral administration of 1g/kg body weight of aqueous stem bark extracts of *Fuerstia africana* to mice for one month caused a significant increase in GGT and CK and a significant decline in ALT, AST, LDH, AMY, T-BIL, D-BIL and Creatinine relative to that of the normal control mice; however, no significant alteration in the levels of Urea and ALP by the same extract dose compared to that of the respective normal control group (Tables 4.14 and 4.15). Intraperitoneal administration of the same dose of aqueous stem bark extracts of *Fuerstia africana* to mice for one month significantly increased the levels of Urea, ALT, GGT, CK, AMY, and T-BIL but did not significantly alter the levels of AST, LDH, ALP, D-BIL and creatinine compared to that of the respective normal control group (Tables 4.16 and 4.17).

Oral administration of 1g/kg body weight of aqueous stem bark extracts of *Aloe secundiflora* to mice for one month caused a significant increase in the level CK and a significant decline in the levels of ALT, AST, ALP, D-BIL and creatinine relative to that of the normal control mice; however, the levels of Urea, GGT, LDH, AMY, and T-BIL were similar to those of the normal control mice (Tables 4.14 and 4.15). Intraperitoneal administration of the same dose of aqueous stem
bark extracts of *Aloe secundiflora* to mice for one month increased the levels of GGT, AMY, ALP, T-BIL and D-BIL but did not significantly alter the levels of Urea, ALT, AST, LDH, CK, and creatinine relative to that of the normal control mice (Tables 4.16 and 4.17).

Oral administration of 1g/kg body weight of aqueous stem bark extracts of *Kleinia squarrosa* to mice for one month caused an increase in the levels of Creatinine and decreased the levels of AST, LDH, CK, and ALP but did not significantly alter the levels of Urea, ALT, GGT, AMY, T-BIL and D-BIL relative to that of the normal control mice (Tables 4.14 and 4.15). Intraperitoneal administration of the same dose of aqueous stem bark extract of *Kleinia squarrosa* to mice caused significant increase in the levels of Urea, ALT, GGT, LDH, T-BIL, creatinine and D-BIL but did not significantly alter the levels of CK, AMY and ALP relative to that of the normal control mice (Tables 4.16 and 4.17).
Table 4.14: The effects of oral administration of 1g/kg body weight of aqueous plant extracts on enzyme levels in mice.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Enzyme Activities</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ALT (U/L)</td>
</tr>
<tr>
<td>Control</td>
<td>132.6±20.6</td>
</tr>
<tr>
<td><em>Acacia nilotica</em></td>
<td>86.2±3.7*</td>
</tr>
<tr>
<td><em>Zanthoxylum africanum</em></td>
<td>66.2±7.5*</td>
</tr>
<tr>
<td><em>Fuerstia africana</em></td>
<td>82.8±9.6*</td>
</tr>
<tr>
<td><em>Aloe secundiflora</em></td>
<td>66.2±10.0*</td>
</tr>
<tr>
<td><em>Kleinia squarrosa</em></td>
<td>132.7±14.1</td>
</tr>
</tbody>
</table>

Results are expressed as Mean ± Standard Deviation (SD) for five animals in each treatment; *p < 0.05 is considered significant when the mean of the control animals is significantly different from that of the extract treated animals by t-test.
Table 4.15: The effects of oral administration of 1g/kg body weight of aqueous plant extracts on the levels of selected metabolites in mice.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Metabolite Levels</th>
<th>CREAT (μmol/L)</th>
<th>T-BIL (μmol/L)</th>
<th>D-BIL (μmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>UREA (mmol/L)</td>
<td>9.6±0.7</td>
<td>75.0±8.5</td>
<td>0.8±0.1</td>
</tr>
<tr>
<td><em>Acacia nilotica</em></td>
<td>8.7±1.0</td>
<td>75.8±8.5</td>
<td>1.2±0.3*</td>
<td>0.5±0.1</td>
</tr>
<tr>
<td><em>Zanha Africana</em></td>
<td>7.4±0.6*</td>
<td>54.4±3.2*</td>
<td>0.5±0.1*</td>
<td>0.3±0.1*</td>
</tr>
<tr>
<td><em>Fuerstia Africana</em></td>
<td>10.6±1.2</td>
<td>49.7±3.8*</td>
<td>0.5±0.1*</td>
<td>0.3±0.1*</td>
</tr>
<tr>
<td><em>Aloe secundiflora</em></td>
<td>8.8±1.0</td>
<td>44.8±2.6*</td>
<td>0.7±0.1</td>
<td>0.2±0.1*</td>
</tr>
<tr>
<td><em>Kleinia squarrosa</em></td>
<td>10.1±0.9</td>
<td>93.9±11.6*</td>
<td>0.9±0.2</td>
<td>0.3±0.1</td>
</tr>
</tbody>
</table>

Results are expressed as Mean ± Standard Deviation (SD) for five animals in each treatment; *p < 0.05 is considered significant when the mean of the control animals is significantly different from that of the extract treated animals by t-test.
Table 4.16: The effects of intraperitoneal administration of 1g/kg body weight of aqueous plant extracts on enzyme levels in mice.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Enzyme Activities</th>
<th>ALT (U/L)</th>
<th>AST (U/L)</th>
<th>GGT (U/L)</th>
<th>LDH (U/L)</th>
<th>CK (U/L)</th>
<th>AMY (U/L)</th>
<th>ALP (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>80.3±7.0</td>
<td>523.2±94.7</td>
<td>2.0±1.0</td>
<td>2137.2±159.4</td>
<td>351.0±59.1</td>
<td>1676.4±230.2</td>
<td>46.6±10.4</td>
</tr>
<tr>
<td>Acacia nilotica</td>
<td></td>
<td>89.4±6.4</td>
<td>368.2±77.1*</td>
<td>1.8±1.3</td>
<td>1935.2±238.4</td>
<td>392.8±46.3</td>
<td>1513.4±189.5</td>
<td>46.0±10.9</td>
</tr>
<tr>
<td>Zanzha africana</td>
<td></td>
<td>98.8±21.5</td>
<td>383.2±48.4*</td>
<td>4.0±1.2*</td>
<td>2087.2±265.8</td>
<td>336.0±92.4</td>
<td>1650.0±209.3</td>
<td>52.6±20.0</td>
</tr>
<tr>
<td>Fuerstia africana</td>
<td></td>
<td>118.0±11.8*</td>
<td>489.6±56.3</td>
<td>3.8±1.3*</td>
<td>1963.4±70.8</td>
<td>655.6±65.8*</td>
<td>3251.6±457.0*</td>
<td>41.8±14.7</td>
</tr>
<tr>
<td>Aloe secundiflora</td>
<td></td>
<td>93.6±13.7</td>
<td>513.6±131.1</td>
<td>3.6±0.6*</td>
<td>2127.4±208.4</td>
<td>396.8±151.4</td>
<td>2397.4±324.3*</td>
<td>62.0±9.5*</td>
</tr>
<tr>
<td>Kleinia squarrosa</td>
<td></td>
<td>367.0±31.3*</td>
<td>1030.2±70.1*</td>
<td>8.6±1.1*</td>
<td>5397.6±161.1*</td>
<td>436.4±40.1</td>
<td>1443.0±106.8</td>
<td>49.6±3.9</td>
</tr>
</tbody>
</table>

Results are expressed as Mean ± Standard Deviation (SD) for five animals in each treatment; *p < 0.05 is considered significant when the mean of the control animals is significantly different from that of the extract treated animals by t-test.
Table 4.17: The effects of intraperitoneal administration of 1g/kg body weight of aqueous plant extracts on the levels of selected metabolites in mice.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Metabolite Levels</th>
<th>CREAT (µmol/L)</th>
<th>T-BIL (µmol/L)</th>
<th>D-BIL (µmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7.2±0.8</td>
<td>43.2±6.1</td>
<td>4.5±1.2</td>
<td>2.6±0.7</td>
</tr>
<tr>
<td><em>Acacia nilotica</em></td>
<td>8.0±0.7</td>
<td>37.0±6.9</td>
<td>5.8±2.1</td>
<td>3.4±1.1</td>
</tr>
<tr>
<td><em>Zanthoxylum fagara</em></td>
<td>8.2±0.8</td>
<td>28.6±3.5*</td>
<td>8.7±0.9*</td>
<td>5.3±0.8*</td>
</tr>
<tr>
<td><em>Fuerstia africana</em></td>
<td>8.6±0.9*</td>
<td>35.4±5.4*</td>
<td>7.0±1.8*</td>
<td>3.8±1.3</td>
</tr>
<tr>
<td><em>Aloe secundiflora</em></td>
<td>7.9±1.1</td>
<td>47.2±10.8</td>
<td>8.9±1.6*</td>
<td>5.3±0.6*</td>
</tr>
<tr>
<td><em>Kleinia squarrosa</em></td>
<td>22.4±0.9*</td>
<td>62.8±3.3*</td>
<td>18.2±1.9*</td>
<td>9.7±1.1*</td>
</tr>
</tbody>
</table>

Results are expressed as Mean ± Standard Deviation (SD) for five animals in each treatment; *p < 0.05 is considered significant when the mean of the control animals is significantly different from that of the extract treated animals by t-test.
4.2.5 Histopathology

Oral administration of 1g/kg body weight of aqueous extracts of the five studied medicinal plants did not cause any significant histopathological lesion on the liver, brain, kidney, lung, spleen, heart and testes when compared to those of the normal control mice. Intraperitoneal administration of same dose of aqueous stem bark extracts of *Kleinia squarrosa* to mice caused significant lesions to the kidney, liver and spleen relative to those of the normal control mice (Plates 4.1, 4.2, 4.3 and 4.4). However, no histopathological lesions were seen in the mice groups intraperitoneally treated with same dose of the other four aqueous plant extracts relative to those of the normal control mice.

The kidney parenchyma of the mice intraperitoneally treated with aqueous stem bark extract of *Kleinia squarrosa* at 1g/kg body weight demonstrated changed appearance in renal cells, with some cells staining pale compared to normal pink staining cells of the normal control mice (Plate 4.1 and 4.2). The renal parenchyma was disorganized with loss of structure. This reflects degeneration of the kidney tubular epithelial cells. The liver of the mice intraperitoneally treated with aqueous stem bark extract of *Kleinia squarrosa* at 1g/kg body weight demonstrated abnormalities such as hepatocytes without their normal columnar arrangement when compared to the liver cells of the normal control mice. There was individualization of hepatocytes and the cytoplasm was pale staining and many hepatocytes were clumped together (Plate 4.3); the spleen of the mice
intraperitoneally treated with aqueous stem bark extract of *Kleinia squarrosa* at 1g/kg body weight demonstrated depopulation of the lymphoid follicles when compared to the spleen of the normal control mice (Plate 4.4).
Plate 4.1: Histological section of a kidney from a normal mouse intraperitoneally treated with 0.1ml physiological saline: (A) the kidney appearance is uniform; mice treated with the aqueous stem bark extract of *Kleinia squarrosa* at a dose level of 1g/kg body weights (25 mg daily) in 0.1ml physiological saline: (B) the kidney architecture is lost. Magnification x 100.
Plate 4.2: Histological section of a kidney from a normal mouse intraperitoneally treated with 0.1ml physiological saline: (A) the kidney appearance is uniform; mice treated with the aqueous stem bark extract of *Kleinia squarrosa* at a dose level of 1g/kg body weights (25 mg daily) in 0.1ml physiological saline: (B). The renal parenchyma appears coagulated with no distinct nephrons. Magnification x 400.
Plate 4.3: Histological section of a liver from a normal mouse intraperitoneally treated with 0.1ml physiological saline: (A) the cells are well arranged and their sizes are uniform; mice treated with the aqueous stem bark extract of *Kleinia squarrosa* at a dose level of 1g/kg body weights (25 mg daily) in 0.1ml physiological saline: (B) the hepatocytes have lost their normal columnar arrangement and many hepatocytes are clumped together. Magnification x 400.
Plate 4.4: Histological section of a spleen from a normal mouse intraperitoneally treated with 0.1ml physiological saline: (A) the lymphoid follicles are well populated; mice treated with the aqueous stem bark extract of *Kleinia squarrosa* at a dose level of 1g/kg body weights (25 mg daily) in 0.1ml physiological saline: (B) the lymphoid follicles are depopulated. Magnification x 400.
4.3 Quantitative analysis of the phytochemical composition of aqueous extracts of the five studied medicinal plants.

The results of quantitative analysis of five major groups of phytochemical constituents in the aqueous plant extracts are shown in table 4.18. *Aloe secundiflora* yielded the highest amount of tannins, followed by *Fuerstia africana*, *Zanha africana*, *Kleinia squarrosa*, and *Acacia nilotica*, respectively. *Acacia nilotica* yielded the highest amount of total phenols followed by *Zanha africana*, *Kleinia squarrosa*, *Fuerstia africana*, and *Aloe secundiflora*, respectively. *Aloe secundiflora* yielded the highest amounts of flavonoids followed by *Kleinia squarrosa*, *Acacia nilotica*, *Fuerstia africana*, and *Zanha africana*, respectively. *Acacia nilotica* yielded the highest amounts of saponins followed by *Aloe secundiflora*, *Zanha africana*, *Kleinia squarrosa*, and *Fuerstia africana*, respectively. *Fuerstia africana* yielded the highest amounts of alkaloids followed by *Kleinia squarrosa*, *Zanha Africana*, *Aloe secundiflora*, and *Acacia nilotica*, respectively.
Table 4.18: Quantitative analysis of the phytochemical composition of aqueous extracts of the five studied medicinal plants

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Phytochemical content</th>
<th>Total Phenols</th>
<th>Flavonoids</th>
<th>Saponins</th>
<th>Alkaloids</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tannins</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acacia nilotica</td>
<td>0.4547±0.058</td>
<td>3.5410±0.220</td>
<td>3.6141±0.226</td>
<td>105.600±9.994</td>
<td>39.180±1.782</td>
</tr>
<tr>
<td>Zanha africana</td>
<td>0.7671±0.004</td>
<td>1.8503±0.077</td>
<td>1.5830±0.342</td>
<td>52.333±3.960</td>
<td>56.520±7.806</td>
</tr>
<tr>
<td>Kleinia squarrosa</td>
<td>0.6975±0.094</td>
<td>1.3823±0.292</td>
<td>4.5998±0.339</td>
<td>45.067±3.489</td>
<td>93.260±18.074</td>
</tr>
<tr>
<td>Fuerstia africana</td>
<td>6.7307±0.262</td>
<td>0.7002±0.091</td>
<td>1.8668±0.221</td>
<td>32.533±7.448</td>
<td>104.500±12.699</td>
</tr>
<tr>
<td>Aloe secundiflora</td>
<td>7.8458±0.243</td>
<td>0.2921±0.083</td>
<td>5.9588±0.323</td>
<td>73.867±5.280</td>
<td>47.120±8.259</td>
</tr>
</tbody>
</table>

Results are expressed as Mean ± Standard Deviation (SD). Tannins and Total Phenols are expressed as mg/g gallic acid equivalent (GAE), flavonoids are expressed as mg/g quercetin equivalent (QE), alkaloids and saponins are in mg/g.
4.4 Mineral Elements Analysis

The mineral composition of the five plants used traditionally in the management of diabetes mellitus is shown in table 4.19. Sodium (Na), chlorine (Cl), potassium (K), Calcium (Ca), Titanium (Ti), Vanadium (V), chromium (Cr), manganese (Mn), iron (Fe), Copper (Cu), Zinc (Zn), Arsenic (As), and Cadmium (Cd) were present at varying quantities in all the five plants (*Acacia nilotica*, *Aloe secundiflora*, *Fuerstia africana*, *Kleinia squarrosa* and *Zanha africana*). Magnesium (Mg) was present at varying quantities in *Aloe secundiflora*, *Fuerstia africana*, *Kleinia squarrosa* and *Zanha africana* and Nickel (Ni) and Lead (Pb) were present at varying quantities in *Acacia nilotica*, *Fuerstia africana*, *Kleinia squarrosa* and *Zanha africana*. The levels of these measured minerals and trace element were all below the recommended daily allowance.
Table 4.19: Mineral levels and amount given to each mouse from the aqueous extracts of the five studied medicinal plants

<table>
<thead>
<tr>
<th>Element</th>
<th>Acacia nilotica</th>
<th>Aloe secundiflora</th>
<th>Fuertisia africana</th>
<th>Kleinia squarrosa</th>
<th>Zanha africana</th>
<th>RDA for mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na</td>
<td>2448.2±275.2</td>
<td>1766.0±242.8</td>
<td>1922.2±437.3</td>
<td>2263.6±119.6</td>
<td>1893.3±128.2</td>
<td>5 x 10^5</td>
</tr>
<tr>
<td></td>
<td>61.205</td>
<td>44.15</td>
<td>48.055</td>
<td>56.59</td>
<td>47.3325</td>
<td>(178.6)</td>
</tr>
<tr>
<td>Mg</td>
<td>&lt;200</td>
<td>221.8±74.2</td>
<td>384.0±145.3</td>
<td>212.2±36.9</td>
<td>205.9±41.7</td>
<td>4.2 x 10^5</td>
</tr>
<tr>
<td></td>
<td>5.545</td>
<td>9.602</td>
<td>5.305</td>
<td>5.1475</td>
<td>5.1475</td>
<td>(150)</td>
</tr>
<tr>
<td>Cl</td>
<td>199.3±4.2</td>
<td>95.58±2.81</td>
<td>687.0±28.4</td>
<td>81.8±1.2</td>
<td>143.2±1.9</td>
<td>7.5 x 10^5</td>
</tr>
<tr>
<td></td>
<td>4.9825</td>
<td>2.3895</td>
<td>17.175</td>
<td>2.045</td>
<td>3.58</td>
<td>(267.9)</td>
</tr>
<tr>
<td>K</td>
<td>416.5±6.5</td>
<td>1107.1±19.8</td>
<td>4301.7±161.8</td>
<td>695.7±6.3</td>
<td>1474.6±13.7</td>
<td>3.5 x 10^6</td>
</tr>
<tr>
<td></td>
<td>10.4125</td>
<td>27.6775</td>
<td>107.5425</td>
<td>17.3925</td>
<td>36.865</td>
<td>(1250)</td>
</tr>
<tr>
<td>Ca</td>
<td>675.5±9.7</td>
<td>190.4±2.3</td>
<td>408.1±16.2</td>
<td>177.3±1.8</td>
<td>50.8±0.6</td>
<td>1.0 x 10^6</td>
</tr>
<tr>
<td></td>
<td>16.8875</td>
<td>4.7605</td>
<td>10.2025</td>
<td>4.4325</td>
<td>1.27</td>
<td>(357.1)</td>
</tr>
<tr>
<td>Ti</td>
<td>11.4±0.3</td>
<td>1.1±0.1</td>
<td>6.4±0.4</td>
<td>3.0±0.08</td>
<td>0.41±0.05</td>
<td>&lt; 1.8 x 10^4</td>
</tr>
<tr>
<td></td>
<td>0.285</td>
<td>0.0275</td>
<td>0.16</td>
<td>0.0765</td>
<td>0.01025</td>
<td>&lt; (0.64)</td>
</tr>
<tr>
<td>V</td>
<td>5.8±0.2</td>
<td>0.3±0.1</td>
<td>2.4±0.2</td>
<td>0.73±0.04</td>
<td>0.16±0.03</td>
<td>3.5 x 10</td>
</tr>
<tr>
<td></td>
<td>0.145</td>
<td>0.0075</td>
<td>0.06</td>
<td>0.01825</td>
<td>0.004</td>
<td>(12.5)</td>
</tr>
<tr>
<td>Cr*</td>
<td>0.000±0.003</td>
<td>0.000±0.003</td>
<td>0.000±0.003</td>
<td>0.000±0.003</td>
<td>0.000±0.003</td>
<td>2.3 x 10^5</td>
</tr>
<tr>
<td></td>
<td>0.000225</td>
<td>0.000225</td>
<td>0.000225</td>
<td>0.000225</td>
<td>0.000225</td>
<td>(0.82)</td>
</tr>
<tr>
<td>Mn</td>
<td>2.0±0.1</td>
<td>6.5±0.2</td>
<td>15.95±0.73</td>
<td>2.26±0.05</td>
<td>2.41±0.05</td>
<td>8.0 x 10^3</td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>0.1625</td>
<td>0.39875</td>
<td>0.0565</td>
<td>0.06025</td>
<td>(2.9)</td>
</tr>
<tr>
<td>Fe</td>
<td>21.52±0.39</td>
<td>14.00±0.33</td>
<td>36.76±1.52</td>
<td>10.18±0.12</td>
<td>16.29±0.19</td>
<td>&lt; 1 x 10^3</td>
</tr>
<tr>
<td></td>
<td>0.538</td>
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<td>0.57±0.02</td>
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<td>(3.9)</td>
<td>3.5 x 10^4</td>
<td>(0.0125)</td>
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</tbody>
</table>

Results on the concentration of each mineral are expressed as µg/g of dry powder of the five studied plants of three determinations and are in the upper row of each plant; the amount of each mineral administered in µg based on its concentration in each plant's extract is in the lower row. This is compared with the recommended daily allowance shown in the last column. This is expressed per 25g, the average weight of each mouse. *Recommended daily allowance estimated from that of human beings stated in Strain & Cashman (2009).
CHAPTER FIVE
DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

The present work was undertaken to *in vivo* investigate the hypoglycemic activity and safety of the aqueous stem bark extracts of *Kleinia squarrosa*, *Acacia nilotica*, *Aloe secundiflora*, and *Fuerstia africana* and aqueous leaf extracts of *Zanha africana* in alloxan-induced diabetic mice. The alloxan-induced diabetic mice had an average of 391% increase in blood glucose levels compared to normal control group.

The study demonstrated that both oral and intraperitoneal administration of the aqueous extracts of the five studied plants had varying degrees of hypoglycemic activity at the four dose levels tested (50mg/kg body weight, 100mg/kg body weight, 200mg/kg body weight, and 300mg/kg body weight) except *Fuerstia africana* which was inactive through the oral route, and demonstrated slight but significant blood glucose lowering effect through the intraperitoneal at a dose level 300 mg/kg body weight only. The hypoglycemic activity demonstrated by the aqueous stem bark extracts of *Kleinia squarrosa* confirmed the study carried out by Murugi *et al* (2012) who reported antidiabetic activity with aqueous extracts of the same plant collected from Embu County, Kenya using the intraperitoneal route at doses of 50-150mg/kg body weight.
The hypoglycemic properties of these extracts could result from the alkaloids, saponins, total phenols, flavonoids, and tannins which are present in these plants. For instance alkaloids collected from leaves of *Acanthus montanus* intraperitoneally administered at doses of 100, 200 and 400mg/kg body weight showed hypoglycemic action in alloxan-induced diabetic rats (Odoh & Ezugwu, 2012). Tiong *et al* (2013) demonstrated that the four indole alkaloids, vindoline I, vindolidine II, vindolicine III, and vindolinine IV, isolated from the leaves of *Catharanthus roseus* which all increased glucose uptake in pancreatic and muscle cells (β-TC6 and C2C12 Cells, respectively) and inhibited PTP-1B, an enzyme that belongs to the protein tyrosine phosphatase family and a negative regulator of the insulin signaling pathway. The alkaloids of *Ephedra distachya* herbs and L-ephedrine have shown antihyperglycemic effect in diabetic mice due to regeneration and restoration of atrophied pancreatic islets that induces the secretion of insulin (Chauhan, 2010).

Saponins have also been reported to be antidiabetic. Saponins are glycosides of triterpenes, steroids or alkaloids. Previous studies have demonstrated the hypoglycemic activity of triterpenoid glycosides (Reher, Slijepcevic & Krans, 1991; Kako *et al*, 1997). Presence of saponins in this extract could also be responsible for the hypoglycemic activity. For instance ginseng and its saponins have been shown to lower blood glucose in alloxan-treated, genetically diabetic, and normal mice (Kimura & Suzuki, 1985). In elderly patients with
hyperglycemia, saponins were shown to reduce serum glucose (Chen & Zhang, 1987). Total saponins from the seeds of *Entada phaseoloides* dramatically reduced fasted blood glucose and alleviated hyperglycemia associated oxidative stress in type 2 diabetic rats (Zheng *et al.*, 2012).

Flavonoids act on various molecular targets and regulate different signaling pathways in pancreatic β-cells, hepatocytes, adipocytes and skeletal myofibers (Babu, Liu & Gilbert, 2013). Several studies have demonstrated the hypoglycemic action of flavonoids using different experimental methods, for instance Lu *et al.* (2010) reported that total flavonoids from *Litsea coreana* leaves showed a significant increase in insulin sensitivity, serum HDL-C level and SOD activities, and also decreased the expression of PTP1B in diabetic rat liver. Flavonoids isolated from the leaves of *ipomoea batatas*, ameliorated blood glucose level and lipid parameters in alloxan induced diabetic mice at 50-150 mg/kg (Li *et al.*, 2009).

Flavonoid fraction from *Pterocarpus marsupium* has been shown to cause pancreatic beta cell regranulation. Epicatechin, its active principle, has been found to be insulinogenic thus enhancing insulin release and conversion of proinsulin to insulin in vitro (Modak *et al.*, 2007). Flavonoid glycosides such as strictinin, isostrictinin and pedunculagin are the effective constituents of *Psidium guajava,*
which have been used in clinical treatment of diabetes due to improved sensitivity of insulin (Chauhan, 2010).

There are two types of tannins; condensed tannins and hydrolysable tannins. Hydrolysable tannins consist of 2 main groups, Tannic acid (TA), also called gallotannins, and ellagitannins (Liu et al., 2005). It was found that condensed tannins extracted from selected Kenyan foods exhibited antidiabetic activity through inhibition of α-amylase and α-glucosidase enzymes (Kunyanga et al., 2011). In addition Liu et al (2005) reported that commercially available tannic acid possessed activities that both stimulated glucose transport and inhibited adipocyte differentiation in 3T3-L1 cells. The same study also demonstrated that TA induced phosphorylation of the insulin receptor (IR) and act, as well as translocation of glucose transporter 4 (GLUT 4), the protein factors involved in the signaling pathway of insulin-mediated glucose transport and also inhibited the expression of key genes for adipogenesis. Tannin stimulates the receptor cells to utilize carbohydrate (Kumari, Jain & Dave, 2014).

Apart from these phytochemical components, the hypoglycemic action of the plants of this study could be related to the presence of trace elements like: chromium (Cr), zinc (Zn), iron (Fe), magnesium (Mg), manganese (Mn), selenium (Se), and vanadium (V). These elements possess antidiabetic activity. For instance the trivalent Cr is a potentiator of insulin action (Dods, 2010). It is a
part of glucose tolerance factor (GTF), a biologically active substance manufactured in the body that regulates glucose biotransformation and increases the number of insulin receptors, enhances receptor binding, and potentiates insulin action (Piero et al., 2012f). It was found that experimental chromium deficiency leads to impaired glucose tolerance, which is improved by chromium supplementations (Mooradian et al., 1994; O'Connell, 2001).

Zinc is virtually involved in all aspects of insulin metabolism: synthesis, secretion and utilization (Piero et al., 2012f). It is a powerful guardian against viral infections, and play role in protecting β-cells from destruction. Type 1 diabetics are often zinc deficient, and zinc supplements have been shown to lower blood sugar levels in some of these cases (Anon, 2014). Magnesium modulates glucose transport across cell membranes and is a cofactor in various enzymatic pathways involved in glucose oxidation (Mooradian et al., 1994). Paolisso et al (1989) showed that the insulin secretory capacity improved with dietary Mg supplementation for 4 weeks.

A deficiency of manganese is common amongst diabetics (Anon, 2014). Experimental evidence suggests that manganese (Mn) deficiency in guinea pigs can cause impaired glucose utilization and Mn supplementation can reverse glucose intolerance induced by Mn deficiency (Mooradian, 1987). Selenium has been shown to mediate a number of insulin-like actions both *in vivo* and *in vitro*. 
These insulin-like actions include stimulating glucose uptake and regulating metabolic processes such as glycolysis, gluconeogenesis, fatty acid synthesis and the pentose phosphate pathway (Stapleton, 2000). Vanadium was used for the control of blood sugar before the discovery of insulin (Piero et al, 2012f). In animal models, vanadium has been shown to facilitate glucose uptake and metabolism, and enhance insulin sensitivity (Cohen et al, 1995; Halberstam et al, 1996; O’Connell, 2001).

Studies in rats and humans have indicated that Nickel deprivation depresses growth, reproductive performance, and plasma glucose and that it alters the distribution of other elements in the body, including calcium, iron, and zinc (Nielson, 2000). Calcium improves insulin sensitivity in some type 2 diabetic populations (Lucy et al, 2002). Potassium supplementation yields improved insulin sensitivity, responsiveness and secretion; insulin administration induces a loss of potassium; and a high potassium intake reduces the risk of heart disease, atherosclerosis, and cancer (Khaw & Barrett-Connor, 1984; Norbiato et al, 1984). Iron influences glucose metabolism and reciprocally, iron influences insulin action. Iron interferes with insulin inhibition of glucose production by the liver (Niederau et al, 1984).

The intraperitoneal administration of all the studied five extracts and oral administration of the Kleinia squarrosa and Zanha africana caused decrease in
growth rate. This decrease in growth rate may have been caused by the presence of alkaloids, saponins, flavanoids, and tannins. Alkaloids such as p-octopamine and synephrines may reduce body weight by exerting adrenergic agonist activity (Tucci, 2010). Synephrines increase energy expenditure (EE) (resting energy expenditure [70%], thermic effect of feeding [10%], and energy expenditure of physical activity [20%]) and decrease food intake in addition to decreasing gastric motility (slows gastric emptying and intestinal transit) and indirectly producing increased feeling of satiety and a decreased appetite (Tucci, 2010). Nicotine an alkaloid induces weight loss by exerting its effects through the central nervous system and metabolic actions by reducing appetite and altering feeding patterns. It increases metabolic rate and increases energy expenditure (EE) and, hence decreases metabolic efficiency (Tucci, 2010).

In the CNS, nicotine modulates the central nervous system pathways that regulate several aspects of food intake (Tucci, 2010). Cathinone (1-a-aminopropiophenone) (monoamine alkaloid) and cathine (D-nor-pseudoephedrine) affect appetite centrally, by acting in the hypothalamus. Cathinone enhances sympathomimetic activity leading to a delay in gastric emptying; Pregnane glycosides have peripheral and central effects. In the adipose tissue, pregnane glycosides reduce lipogenesis. In the central structures regulating appetite, pregnane glycosides and their related molecules act by amplifying the signaling of the energy sensing function in the hypothalamus (Tucci, 2010).
The flavanoid, chlorogenic acid reduces body weight by inducing reduction in body fat by reducing the absorption of glucose (energy source) leading to an increase in the consumption of fat reserves. A major consequence of blocking digestion of carbohydrates in the proximal gut is colonic fermentation which leads to increased microbial production of gas in the bowl; gas production limits glucose utilization (Tucci, 2010). Catechins (flavanoids) such as epigallocatechin gallate, epigallocatechin, epicatechin gallate, and epicatechin are associated with increase in sympathetic nervous system activity, thermogenesis, and fat oxidation and hence accounting for the reduced body weight (Tucci, 2010).

Catechins also induce reduction of body fat by inhibiting small intestine micelle formation and inhibiting α-glucosidase activity leading to decreased carbohydrate absorption (Tucci, 2010). Caffeine, a flavanoid induces weight loss by decreasing energy intake (EI) by acting through the central and peripheral nervous system mechanisms and by promoting thermogenesis and lipolysis (Tucci, 2010). The central nervous system effects of caffeine are due to its effects on the widely distributed adenosine α1, α2A, and α2B receptors (Tucci, 2010).

Forskolin, a terpenoid reduces body weight by acting on adenylate cyclase that converts ATP to cyclic adenosine monophosphate (cAMP). Cyclic adenosine monophosphate (cAMP) promotes lipolysis, increases the body's basal metabolic
rate and increases use of body fat, and protein degradation and/or decreases protein synthesis (Tucci, 2010).

For the oral route, tannins which are present in high amounts in *Z. africana* and *K. squarrosa* extracts may reduce feed intake by decreasing palatability and by reducing feed digestion. Palatability is reduced because tannins are astringent. Astringency is the sensation caused by the formation of complexes between tannins and salivary glycoproteins. Low palatability depresses feed intake. Digestibility reduction negatively influences intake because of the filling effect associated with undigested feedstuff (Reed, 1995). Tannins are divided into two: hydrolysable and condensed tannins. Hydrolysable tannins are converted by microbial metabolism and gastric digestion into absorbable low molecular weight metabolites such as tannic acid which are toxic. The major lesions associated with hydrolysable tannins poisoning are hemorrhagic gastroenteritis which decreases absorption of nutrients and necrosis of the liver and kidney (Reed, 1995).

Protanthocyanidins (PAs) (condensed tannins) retard growth by inhibiting feed intake and digestibility. Protanthocyanidins (PAs) which are not absorbed by the digestive tract, damage the mucosa of the gastrointestinal tract, decreasing the absorption of nutrients such as proteins, carbohydrates and essential amino acids such as methionine and lysine. They also increase excretion of proteins and
essential amino acids and alter the excretion of certain cations (Glick & Joslyn, 1969).

The reduced growth rates of the experimental mice intraperitoneally administered with 1g/kg body weight relative to the control mice correlates with the reduced percent organ to body weight of liver (*Kleinia squarrosa* orally administered) and testes (*Acacia nilotica* intraperitoneally administered). Normalization of organ weight to body weight helps eliminate variations due to body weight differences as some apparent difference in organ weight might have been the result of differences in organ weight which are unrelated to the treatment (Sellers *et al.*, 2007). The increased percent organ to body weight of liver (*Kleinia squarrosa* and *Zanha africana*) Brain (*Zanha africana, Fuerstia africana*, and *Aloe secundiflora*), kidneys (*Zanha africana*) and Lungs (*Kleinia squarrosa*) of mice intraperitoneally administered with aqueous extracts at 1 g/kg body weight daily for one month could not be explained in this study. It is possible that the extracts promoted higher metabolic activity in these organs.

The investigated hematological parameters in this study are useful in the assessment of the toxic potentials of the plant extracts in mammals and human beings. They provide information about the status of bone marrow activity and hemolysis (Iniaghe, Egharevba & Oyewo, 2013). The dramatic reduction of red blood cell count and packed cell volume observed in mice administration with
aqueous extracts of *K. squarrosa* at 1g/kg body weight relative to the control mice indicates induction of anemia by these extracts. This anemic state leads to tissue hypoxia. These abnormal blood conditions could be caused by toxic constituents in these plants extracts including among others total phenols, alkaloids, saponins, flavanoids, and tannins present in these extracts. Saponins hemolyse and cause cell death in many tissues (Al-Sultan, Hussein & Hegazy, 2000; Diwan, Abdel-Hassan & Mohammed, 2000).

Alkaloids have been shown to cause liver megalocytosis, proliferation of biliary tract epithelium, liver cirrhosis and nodular hyperplasia (Zeinsteger *et al*, 2003). Terpenoids have been shown to increase membrane permeability to divalent and monovalent ions (Zeinsteger *et al*, 2003). These extract constituents could be reducing the erythron parameters (Barger, 2003). This toxicity may not have been due to the presence of trace elements/minerals since the amounts administered into each mouse daily at a dose of 1g/kg body weight were below the recommended daily allowance.

Tissue hypoxia causes most tissues to initially enlarge and as the swollen cells continue rupturing, the organ size reduces (organ atropy) (Voet & Voet, 2004). During tissue hypoxia, cells which rely only on glycolysis for ATP production rapidly deplete the store of phosphocreatine (a source of rapid ATP production) and glycogen. As the rate of ATP production decreases below the level required
by membrane ion pumps for the maintenance of proper intracellular ionic concentrations, the osmotic balance of the cell is disrupted so that the cell and its membrane enveloped organelles swell. The overstretched membrane becomes permeable thereby leaking their enclosed contents. The decreased intracellular pH that accompanies anaerobic glycolysis because of lactic acid production permits the released lysosomal enzymes which are only active at acidic pH to degrade the cell contents. Among the degraded components are the initially elevated serum enzymes which are later reduced to values below the control values. The reduced metabolic activity results in irreversible cell damage (Voet & Voet, 2004).

Injury of organs was demonstrated by the enlargement of the liver, kidney, lungs, and brain; histologically this was demonstrated by observations that the spleen, liver, and kidney of mice intraperitoneally treated with aqueous extracts of *K. squarrosa* at 1g/kg body weight daily for one month had depopulation of lymphoid follicles, and loss of liver and kidney architecture; biochemically an increase in levels of serum alanine aminotransferase (liver), aspartate aminotransferase (liver, kidneys, heart and pancreas), γ-glutamyltransferase (liver), lactate dehydrogenase (liver, kidney and heart), creatine kinase (heart and skeletal muscle) and amylase (pancreas) was noted and levels of urea, creatinine, total bilirubin and direct bilirubin fluctuated abnormally.
The decreased levels of platelet count (thrombocytopenia) in mice intraperitoneally administered with 1g of aqueous extract of *K. squarrosa* per kg body weight daily for 28 days may either be associated with (the immune system that causes antibodies produced by the spleen to kill platelets) bone marrow injury caused by toxic phytochemical substances in the extracts.

The decreased levels of white blood cell count and decreased neutrophil, lymphocytes, eosinophils, and monocytes observed in mice intraperitoneally administered with 1g of aqueous plant extracts of *K. squarrosa* per kg body weight daily for 28 days indicate a reduced ability of the body to respond to infection (Kaushansky 1995, Li, Xia & Kuter, 1999) either due to liver or spleen or bone marrow injury caused by toxic phytochemicals contained in this extract.

Liver and spleen injury caused by intraperitoneal treatment with aqueous extracts of *K. squarrosa* at 1g/kg body weight daily in mice for one month is demonstrated by lymphoid follicles depopulation and the presence of hepatocytes without columnar arrangement and some with enlarged hepatocytes. Reduced levels of neutrophils and monocytes imply impaired ability to protect the body against bacteria and eat up small particles of foreign matter; reduced lymphocyte levels imply impaired antibody production against foreign organisms and protection against viruses and fighting cancer; and reduced eosinophils imply impaired ability to kill parasites and impaired allergic responses.
The significant increase in white blood cells observed on intraperitoneal administration of plant extracts of *Z. africana* indicates a more accelerated production of these cells and a boosted immunity to mice by this extract (Kaushansky, 1995; Li, Xia & Kuter, 1999). This could be due to tissue damage caused by some constituents of this plant extract. This argument is in line with the observed enlargement of the liver, brain and kidney and the decrease activity of alanine (oral and IP) and aspartate aminotransferase and an increase in the levels of γ-glutamyltransferase (oral and IP), lactate dehydrogenase and creatine kinase and a reduction in the levels of urea, creatinine (oral and IP), total bilirubin (oral and IP) and direct bilirubin (oral and IP) in mice administered with 1g of *Z. africana* extracts per kg body weight. The observed significant increase in lymphocytes (main effectors cells of the immune system) on intraperitoneal administration of aqueous extracts of *Z. africana* at 1g/kg body weight in mice for 28 days indicates a possible stimulatory effect by these extracts on lymphocyte production.

The observation that orally administered aqueous extracts of *K. squarrosa* at 1g/kg body weight in mice for 28 days caused no histopathological abnormalities compared to the same dose intraperitoneally administered confirms the known fact of the lesser toxicity of drugs administered orally due to poor absorption, protein binding or metabolism in the gastrointestinal tract.
The observation of a general reduction in weekly weight gain, increase in percent organ to body weight in mice intraperitoneally administered with five plants aqueous extracts at 1g/kg body weight daily for 28 days relative to extracts from two plants when orally administered at same dose to biochemical and hematological abnormalities for all the tissues used in this study without obvious histopathologies in all the plant extracts administered may suggest that the effects were subclinical without overt morphological cellular changes discernible at histopathology.

5.2 Conclusion

1- Aqueous extracts of *Kleinia squarrosa, Acacia nilotica, Zanha Africana* and *Aloe Secundiflora* showed hypoglycemic activity in alloxan induced diabetes mice.

2- The intraperitoneal route was more effective in reducing the blood glucose levels than the oral rout except for *Aloe secundiflora*.

3- The aqueous extract of each of the studies plants at high dose of 1g/kg body weights which is far from the therapeutic dose tends to cause toxicological effects. This was well demonstrated in the body and organ weight changes, hematological, and biochemical parameters together with the histopathological analysis.

4- In the toxicological studies the oral administration of the high dose (1g/kg body weight) was found to have less toxic effects than the intraperitoneal
administration of the same dose. This explains why the oral route is the most preferred route by the traditional health practitioners.

5- The antidiabetic action of the studied plants may have resulted from their phytochemical and mineral constituents.

6- The toxic effect of the aqueous extracts of the studied plants at dose of 1g/kg body weight could be associated with their phytochemical constituents and the presence heavy metals like arsenic, lead and Cadmium e.t.c.

5.3 Recommendations

1- This study recommends continues use of the aqueous extracts of *Kleinia squarrosa, Acacia nilotica, Zanha Africana and Aloe Secundiflora* as traditional medicine in the management of diabetes mellitus.

2- Development of easy-to-consume products of these plant extracts such as capsules or tablets to increase their useful period and avoid toxic fermentation products. This will avail crude drugs that are cheaper and affordable to the local population.

3- To undertake further research on these plants to establish the mechanism of their antidiabetic activity.

4- To undertake a combination therapy study of the five studied medicinal plant. As the combination therapy is the applied method in traditional
treatments. This kind of study may produce a combination with higher activity and lesser toxicity.

5- To check the antidiabetic and toxic effect of the organic extracts of these plants and compare that with the findings of the aqueous extracts, in order to find more effective and safer preparations for the use of these plants in the traditional management of diabetes.

6- To isolate the different phytochemicals presents in these plants and subject them into the bioassay and compare their effects with effect of the crude extracts.

7- Undertake preclinical studies on higher animals prior to human clinical trials to authenticate the use of these medicinal plant extracts as herbal remedies for diabetes mellitus.

8- To increase the public awareness on the toxic effects of using the high doses of these plants especially *Kleinia squarrosa*. 
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properties of alkaloids from *Catharanthus roseus* (L.) G. Don. *Molecules*, 18, 9770-9784.


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APPENDICES

Appendix 1: The effects of oral administration of 1g/kg body weight of aqueous plant extracts on organ weights in mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Liver</th>
<th>Brain</th>
<th>Kidney</th>
<th>Lungs</th>
<th>Spleen</th>
<th>Heart</th>
<th>Testes</th>
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</thead>
<tbody>
<tr>
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<td>1.51±0.21</td>
<td>0.57±0.04</td>
<td>0.37±0.05</td>
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<td>0.11±0.02</td>
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<td>1.38±0.08</td>
<td>0.57±0.04</td>
<td>0.33±0.03</td>
<td>0.32±0.04</td>
<td>0.20±0.04</td>
<td>0.10±0.03</td>
<td>0.13±0.03*</td>
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<td>0.43±0.10</td>
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<td>0.44±0.12</td>
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<td>Kleinia squarrosa</td>
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<td>0.22±0.05</td>
<td>0.10±0.03</td>
<td>0.17±0.05</td>
</tr>
</tbody>
</table>

Organ weight is measured in g. Results expressed as Mean ± Standard Deviation (SD) for five animals for each parameter; *p < 0.05 is considered statistically significant when the mean of the experiment group for each plant extract is compared to its relevant control group by t-test.
Appendix 2: The effects of intraperitoneal administration of 1g/kg body weight of aqueous plant extracts on organ weights in mice.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Organ weight</th>
<th>Liver</th>
<th>Brain</th>
<th>Kidney</th>
<th>Lungs</th>
<th>Spleen</th>
<th>Heart</th>
<th>Testes</th>
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<td>1.18±0.22</td>
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<td>0.33±0.08</td>
<td>0.32±0.12</td>
<td>0.15±0.03</td>
<td>0.12±0.04</td>
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<td>1.16±0.10</td>
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<td>1.37±0.27</td>
<td>0.43±0.05</td>
<td>0.35±0.07</td>
<td>0.33±0.10</td>
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<td>0.31±0.07</td>
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<td>0.11±0.05</td>
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<td>0.23±0.03</td>
<td>0.08±0.05*</td>
<td>0.10±0.03</td>
<td>0.16±0.01</td>
</tr>
<tr>
<td>Kleinia squarrosa</td>
<td></td>
<td>1.31±0.16</td>
<td>0.30±0.13</td>
<td>0.41±0.18</td>
<td>0.42±0.05</td>
<td>0.13±0.04</td>
<td>0.13±0.03</td>
<td>0.13±0.02*</td>
</tr>
</tbody>
</table>

Organ weight is measured in g. Results expressed as Mean ± Standard Deviation (SD) for five animals for each parameter; *" p < 0.05 is considered statistically significant when the mean of the experiment group for each plant extract is compared to its relevant control group by t-test.