EFFICACY AND PHYTOCHEMICAL SCREENING OF SELECTED PLANTS USED IN MANAGEMENT OF DIABETES MELLITUS IN MACHAKOS, KENYA

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DECLARATION

This thesis is my original work and has not been presented in any other institution for examination or any other purposes.

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DEDICATION

To my parents Mr. and Mrs. Kimani, my brother Kiarie and sisters Njambi, Wairimu and Nyanjau, thank you for the love.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Diabetes is a problem in Kenya and many herbal preparations are being used to treat it. This study aimed at documenting the plants that are used for the treatment of diabetes mellitus in Machakos County in Kenya, the three most commonly used plants were subjected to phytochemical screening and efficacy evaluation as well as effects on biochemical parameters, liver and kidney histology. The ethnobotanical information was collected through questionnaires, focus group discussions, collection and identification of the plant specimens. Phytochemical screening was done using standard techniques. The most commonly employed species *Zanthoxylum chalybeum*, *Ficus sycomorus* and *Ximenia americana* were selected for phytochemical analysis and efficacy and safety evaluation. Antidiabetic efficacy was determined using a rat model of diabetes mellitus. The efficacy study used 75 adult male Wistar rats. Aqueous stem bark extracts of the three plants were administered to diabetic rats after induction of diabetes via single streptozotocin injection (45mg/kg bwt intraperitoneally). Development of hyperglycemia was assessed by measuring blood glucose three days post induction and comparing these with normal controls. The efficacy of the plant extracts was also compared against Glibenclamide, a conventional diabetes drug.

A total of nineteen plant species distributed across 13 families were identified as being used to manage diabetes mellitus. The secondary metabolites in *Zanthoxylum chalybeum*, *Ficus sycomorus* and *Ximenia americana* were flavonoids, terpenoids, tannins and glycosides. *Zanthoxylum chalybeum* also contained alkaloids and saponins. All three plants investigated

exhibited significant antidiabetic activity compared to the untreated diabetic controls ($P<0.05$). Diabetic rats exhibited elevated fasting blood glucose levels, decreased body weight, and increased water and food intake. *Zanthoxylum chalybeum* stem bark extract decreased fasting blood glucose in diabetic rats at three dose levels (10mg, 100mg and 1000mg). There was no significant difference between the extract fed diabetic rats and the normal controls ($P<0.05$). *Ficus sycomorus* stem bark extract significantly reduced glucose levels in diabetic rats ($P<0.05$) at doses of 100mg and 10mg/kg bwt compared to untreated diabetic rats. *Ximenia americana* stem bark extract at the three dose levels employed, reduced blood glucose to levels that were not statistically significant ($P<0.05$) compared to the Glibenclamide group. Additionally at 100mg and 10mg/kg bwt, blood glucose levels were significantly reduced compared to the untreated diabetic group.

These observations suggest that the aqueous stem bark extracts of *Z. chalybeum*, *F. sycomorus* and *X. americana* possess significant antihyperglycemic activity. The phytochemical composition of the plants may account for the antidiabetic activity observed, as well as the differences in efficacy between the plants. There was no difference in the biochemical parameters in the experimental groups thus the plants can be deemed safe at the dosages used. This study thus validates the traditional use of the three plants for the management of diabetes mellitus in the study area. The study recommends further studies to determine the most efficacious doses of the plant extracts. A study of the remaining sixteen plants should also be carried out to determine their efficacy in the management of diabetes.

CHAPTER ONE

INTRODUCTION

The term diabetes mellitus describes a metabolic disorder of multiple aetiology characterized by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both (DIAMOND Project Group, 2006). The twenty first century has seen a rise in diabetes and its complications in Africa and it is estimated that from 2007 to 2025, the prevalence of diabetes in the continent will increase from 3.1% to 3.5% which is an increase from 10.4 to 18.7 million people (King *et al.*, 1998; Zimmet *et al.*, 2001; Awah *et al*., 2009). The most affected are between the ages of 35 and 64 years impacting people in their most economically productive years (Zimmet *et al.*, 2001). According to the World Health Organization (WHO), the global prevalence was estimated at 2.8% in 2000, with projections of 4.8% in 2030. The total number of persons affected will rise from 171 million in 2000 to 366 million in 2030 if no action is taken (Wild *et al.*, 2004). However, recent data from the International Diabetes Federation (IDF) revealed that this number had already been reached in 2011. The IDF expects an even higher number of 552 million affected persons in 2030 (IDF, 2007; Friedrich, 2012).

The effects of diabetes mellitus include long– term damage, dysfunction and failure of various organs. Diabetes mellitus may present with characteristic symptoms such as thirst, polyuria, blurring of vision, and weight loss. In its most severe forms, ketoacidosis or a non–ketotic hyperosmolar state may develop and lead to stupor, coma and even death in absence of effective

treatment. The long–term effects of diabetes mellitus include progressive development of the specific complications of retinopathy with potential blindness, nephropathy that may lead to renal failure, and/or neuropathy with risk of foot ulcers, amputation, charcot joints, and features of autonomic dysfunction, including sexual dysfunction. People with diabetes are at increased risk of cardiovascular, peripheral vascular and cerebrovascular disease (WHO, 1999; Brownlee, 2005).

The only available pharmacological intervention for Type 1 diabetes is insulin (Gough and Narendran, 2010). Conversely, therapeutic strategies for type 2 diabetes involve insulin and four main classes of oral antidiabetic agents that stimulate pancreatic insulin secretion (sulphonylureas and rapid-acting secretagogues/insulinotropics like glibenclamide, glipizide, rapaglinide), reduce hepatic glucose production (biguanides like metformin), delay digestion and absorption of intestinal carbohydrate (α-glucosidase inhibitors like acarbose) or improve insulin action (thiazolidinediones like pioglitazone, rosiglitazone). Each of above agents suffers from generally inadequate efficacy and number of serious adverse effects (Grant, 2003; Bailey, 2008). With regard to these limitations, there is need to explore other treatment and management strategies (Pareek *et al*., 2009).

The field of herbal medicines research has been gaining significant importance in the last few decades and the demand to use natural products in the treatment of diabetes is increasing worldwide. The available literature shows that there are more than 400 plant species showing

antidiabetic activity (Grover *et al.*, 2002). Although some of these plants have great reputation in traditional medicine, many remain to be scientifically proven (Dropkin, 2010; Kolling *et al.*, 2010). A scientific investigation of traditional herbal remedies for diabetes may provide valuable leads for the development of alternative drugs and strategies.

The streptozotocin-induced Wistar rat model for type 1 diabetes is a good model that has been used extensively in animal models to study both the pathology of diabetes mellitus and complications related to the disease as well as possible interventions.

With the impact of type 2 diabetes set to continue, the risk of related complications such as blindness, amputations and kidney disease are increasing, forcing additional burden on countries already stretched to the limit by common life-threatening infections. Further, the cost of medication, especially the high cost of insulin, is a major handicap to proper diabetes care in Sub-Saharan Africa (Sobngwi *et al.*, 2001; Beran *et al.*, 2005; International Insulin Foundation, 2005). This high cost of anti-diabetic drugs has seen these populations turn to traditional medicines and natural products to treat diabetes. However, for majority of the products, the pharmacology and efficacy has not been studied. There is, therefore, a need to generate efficacy and safety data for these herbal remedies. This information will inform the development of cheaper, anti-diabetic drugs with fewer side effects that will find greater affordability with diabetes patients, particularly those in Africa (Gunjan *et al.*, 2011).

This study investigated the plants used for the management of diabetes in selected locations of Machakos County. Three most commonly used herbal anti-diabetics were subjected to phytochemical screening and efficacy evaluation in Streptozotocin (STZ) - induced diabetes mellitus in Wistar rats.

CHAPTER TWO

LITERATURE REVIEW

2.1 Aetiology and pathogenesis of diabetes mellitus

There are 2 major types of diabetes, type 1 and type 2. Type 1 diabetes accounts for 5-10% of total cases and is characterized by autoimmune or idiopathic beta-cell destruction, usually leading to absolute insulin deficiency. Type 2 accounts for 90-95% of total diabetes cases and may range from predominantly insulin resistance with relative insulin deficiency to a predominantly secretory defect with or without insulin resistance (WHO, 1999).

Glucose, a fundamental source of cellular energy, is released by the breakdown of endogenous glycogen stores that are primarily located in the liver. Glucose is also released indirectly in the muscle through intermediary metabolites. These whole-body energy stores are replenished from dietary glucose, which, after being digested and absorbed across the gut wall, is distributed among the various tissues of the body (Bryant *et al.*, 2002). Although glucose is required by all cells, its main consumer is the brain in the fasting or "postabsorptive" phase, which accounts for approximately 50% of the body's glucose use. Another 25% of glucose disposal occurs in the splanchnic area and the remaining 25% takes place in insulin-dependent tissues, including muscle and adipose tissue (DeFronzo, 2004). Approximately 85% of endogenous glucose production is derived from the liver, with glycogenolysis and gluconeogenesis contributing equally to the basal rate of hepatic glucose production. The remaining 15% of glucose is produced by the kidneys (DeFronzo, 2004).

Normally, following glucose ingestion, the increase in plasma glucose concentration triggers insulin release, which stimulates splanchnic and peripheral glucose uptake and suppresses endogenous glucose production. In healthy adults, blood glucose levels are tightly regulated within a range of 70 to 99 mg/dL, and maintained by specific hormones including insulin, glucagon and incretins as well as the central and peripheral nervous system, to meet metabolic requirements (Tortora and Grabowski, 2003; Guyton and Hall, 2006; Wardlaw and Hampl, 2007). Various cells and tissues such as within the brain, muscle, gastrointestinal tract, liver, kidney and adipose tissue are also involved in blood glucose regulation by means of uptake, metabolism, storage and excretion (Gerich, 2000; Tortora and Grabowski, 2003; DeFronzo, 2004; Guyton and Hall, 2006). This highly controlled process of glucose regulation is evident in the postprandial period, during which under normal physiologic circumstances, glucose levels rarely rise beyond 140 mg/dL even after consumption of a high-carbohydrate meal (Guyton and Hall, 2006).

Among the various hormones involved in glucose regulation, insulin and glucagon, both produced in the pancreas by islets of Langerhans, are the most relevant (Tortora and Grabowski, 2003). Within the islets of Langerhans, β-cells produce insulin and α-cells produce glucagon. Insulin, a potent antilipolytic hormone, reduces blood glucose levels by accelerating transport of glucose into insulin-sensitive cells and facilitating its conversion to storage compounds via glycogenesis and lipogenesis (Tortora and Grabowski, 2003).

Glucagon is produced in response to low normal glucose levels or hypoglycemia and acts to increase glucose levels by accelerating glycogenolysis and promoting gluconeogenesis (Tortora and Grabowski, 2003). After a glucose-containing meal, however, glucagon secretion is inhibited by hyperinsulinemia, which contributes to suppression of hepatic glucose production and maintenance of normal postprandial glucose tolerance (Tortora and Grabowski, 2003). The hormone amylin contributes to reduction in postprandial glucagon, as well as modest slowing of gastric emptying (Drucker and Nauck, 2006).

Incretins, which include glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1), are also involved in regulation of blood glucose, in part by their effects on insulin and glucagon (Porte *et al.*, 2003; Drucker and Nauck, 2006). However, both GLP-1 and GIP are glucose-dependent hormones secreted only when glucose levels rise above normal fasting plasma glucose levels and they do not directly stimulate insulin secretion. Normally, these hormones are released in response to meals and, by activating G protein–coupled receptors on pancreatic β-cells, they aid in stimulation of insulin secretion. However, when glucose levels are low, GLP-1 and GIP levels are diminished (Drucker, 2006).

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia. The characteristic state of chronic hyperglycemia is associated with long-term damage, dysfunction, and potential failure of different organs, especially the eyes, kidneys, nerves, heart, and blood vessels (American Diabetes Association, 2010). Numerous factors contribute to the development

of type 2 diabetes mellitus, with the central defects being inadequate insulin secretion and/ or diminished tissue responses to insulin at one or more points in the complex pathways of hormone action (American Diabetes Association, 2010). Insulin deficiency and insulin resistance frequently coexist, though the contribution to hyperglycemia can vary widely along the spectrum of type 2 diabetes mellitus.

2.2 Complications of diabetes mellitus

Diabetes predisposes patients to opportunistic infections, vascular and neural pathologies. Based on its pathophysiology diabetes mellitus can be acute or chronic.

2.2.1 Acute complications

These include diabetic keto acidosis (DKA) and non-ketotic hyper-osmolar state (NKHS). Diabetic ketoacidosis is seen primarily in individuals with type 1 diabetes mellitus while non-ketotic hyperosmolar state is prevalent in individuals with type 2 diabetes mellitus. The two disorders are associated with absolute or relative insulin deficiency, volume depletion and altered mental state (Kitabchi *et al*., 2008). In DKA, insulin deficiency is combined with counter-regulatory hormone excess with respect to glucagon, catecholamines, cortisol and growth hormone (Stentz *et al*., 2004). The decreased ratio of insulin to glucagon promotes gluconeogenesis, glycogenolysis and ketone body formation in the liver and also increases free fatty acid and amino-acid delivery from fat and muscle to the liver (Hansen and Moller, 2010). Ketosis results from a marked increase in free fatty acid release from adipocytes due to increased

lipolysis (Keller *et al*., 2003). In DKA, nausea and vomiting are often present. Severe DKA may result in lethargy and central nervous system depression eventually leading into coma. Cerebral edema, an extremely serious complication, is seen most frequently in children (Moller *et al*., 2005; Kitabchi *et al*., 2008).

Non-ketotic hyper-osmolar state is most commonly seen in elderly individuals with type 2 diabetes mellitus. Its most prominent features include polyuria, postural hypotension, and a variety of neurological symptoms including altered mental state, lethargy, seizure and possibly coma (Kitabchi *et al*., 2008). Insulin deficiency and inadequate fluid intake are the underlying causes of NKHS. Hyperglycemia consequent to insulin deficiency induces an osmotic diuresis leading to excessive intravascular volume depletion (Gaglia *et al*., 2004).

2.2.2 Chronic complications

The chronic complications of diabetes mellitus affect many organ systems and are responsible for most of the morbidity and mortality associated with the disease. Chronic complications can be either vascular or nonvascular. Vascular complications are then subdivided into microvascular (retinopathy, neuropathy and nephropathy) and macrovascular complications (coronary artery disease, peripheral vascular disease and cerebrovascular disease) (Tripathi and Srivastava, 2006; Fowler, 2008). Nonvascular complications include gastroporesis, sexual dysfunction and skin changes. Diabetes mellitus is the most common cause of adult blindness, a variety of debilitating neuropathies, and cardiac and cerebral disorders (Tripathi and Srivastava, 2006).

Early in the course of diabetes, intracellular hyperglycemia causes abnormalities in blood flow and increased vascular permeability. This reflects decreased activity of vasodilators such as nitric oxide, increased activity of vasoconstrictors such as angiotensin II and endothelin-1 and production of permeability factors such as vascular endothelial growth factor. In diabetes, arterial endothelial dysfunction seems to involve both insulin resistance specific to the phosphotidylinositol–3-OH kinase pathway and hyperglycemia (Cha *et al*., 2000).

2.2.2.1 Diabetic retinopathy

Diabetic retinopathy occurs in 75% of all persons having diabetes for more than 15 years and is the most common cause of blindness (Tripathi and Srivastava, 2006, Fowler, 2008). The risk of developing diabetic retinopathy or other microvascular complications of diabetes depends on the duration and severity of hyperglycemia (Harding *et al*., 2003; Wilkinson *et al*., 2003; Emanuele *et al*., 2005). Diabetic retinopathy is classified into two stages, non-proliferative and proliferative. The non-proliferative stage appears late in the first decade or early in the second decade of disease and is marked by retinal vascular microaneurysms, small hemorrhages in the middle layers of the retina, and cotton-wool spots and includes loss of retinal pericytes, retinal oedema resulting from increased retinal vascular permeability, alterations in regional blood flow, and abnormal retinal microvasculature, all of which lead to retinal ischemia (Gardiner *et al*., 2007). Proliferative retinopathy is characterized by the formation of new blood vessels on the surface of the retina in response to retinal hypoxia. The newly formed vessels may appear at the optic nerve and/or macula and rupture easily, leading to vitreous hemorrhage, fibrosis and retinal

detachment resulting in blindness (Stratton *et al*., 2001**;** Harding *et al*., 2003; Wilkinson *et al*., 2003; Bunce and Wormald, 2006).

2.2.2.2 Neuropathy

The precise nature of injury to the peripheral nerves from hyperglycemia is not known but likely is related to mechanisms such as polyol accumulation, injury from advanced glycosylated end products and oxidative stress. Peripheral neuropathy in diabetes may manifest in several different forms, including sensory, focal/multifocal and autonomic neuropathies. About half of all people with diabetes have some degree of neuropathy, which can be polyneuropathy, mono-neuropathy and/or autonomic neuropathy (Shaw *et al*., 2003). Polyneuropathy is the most common form of neuropathy in diabetes (Tripathi and Srivastava, 2006; Fowler, 2008). There is loss of peripheral sensation which, when coupled with impaired microvascular and macrovascular junction in the periphery, can contribute to non-healing ulcers, the leading cause of non-traumatic amputation (Abbott *et al*., 1998). There is thickening of axons, decrease in microfilaments, and capillary narrowing involving small myelinated or non-myelinated C-fibers. It can occur both from direct hyperglycemia-induced damage to the nerve parenchyma and from neuronal ischemia leading to abnormalities of microvessels, such as endothelial cell activation, pericyte degeneration, basement membrane thickening and monocyte adhesion (Vincent *et al*., 2005; Sorensen *et al*., 2006).

Pure sensory neuropathy is relatively rare and associated with periods of poor glycemic control or considerable fluctuation in diabetes control. It is characterized by isolated sensory findings without signs of motor neuropathy. Symptoms are typically most prominent at night. Mono-neuropathy is less common than polyneuropathy and includes dysfunction of isolated cranial or peripheral nerves. Autonomic neuropathy can involve multiple systems, including cardiovascular, gastrointestinal, genitourinary, sudomotor and metabolic systems (Yagihashi *et al*., 2007; Fowler, 2008).

2.2.2.3 Nephropathy

Diabetic nephropathy is defined by proteinuria greater than 500 mg in 24 hours, but this is preceded by lower degrees of proteinuria, or "microalbuminuria." Microalbuminuria is defined as albumin excretion of 30–299 mg per 24 hours (Fowler, 2008). Without intervention, diabetic patients with microalbuminuria typically progress to proteinuria with decreased glomerular filtration rate and end stage renal failure (Diabetes Control and Complications Trial (DCCT) Research Group, 1995; Giorgino *et al*., 2004). This progression occurs in both type 1 and type 2 diabetes. Dysfunction of the glomerular filtration is attributed to changes in synthesis and catabolism of various glomerular basement membrane macromolecules such as collagen and proteoglycans, leading to an increase in glomerular basement thickening (Microalbuminuria Collaborative Study Group, 1999; Chaturvedi *et al*., 2001). Another possible mechanism to explain the increase in permeability of the glomerulus is the increase in renal vascular

endothelial growth factor (VEGF) levels observed in preclinical models of diabetes, since VEGF is both an angiogenic and a permeability factor (Cha *et al*., 2000).

2.2.2.4 Cardiovascular disorders

In diabetes mellitus there is marked increase in several cardiovascular diseases, including peripheral vascular disease, congestive heart failure, coronary artery disease, myocardial infarction and a one to fivefold increase in sudden death. Cardiovascular disease is the primary cause of death in people with either type 1 or type 2 diabetes (Turner *et al*., 1996; Califf *et al*., 2008; Holman *et al*., 2008).

Macrovascular complications may be unaffected or even worsened by diabetes therapies. An improvement in the lipid profiles of individuals in the intensive group (lower total and low-density lipoprotein cholesterol, lower triglycerides) suggested that intensive therapy may reduce the risk of cardiac vascular mortality. In addition to coronary artery disease, cerebrovascular disease is increased in individuals with diabetes mellitus (Almdal *et al*., 2004; Kissela *et al*., 2005). Individuals with diabetes mellitus have increased incidence of congestive heart failure the etiology of which may include factors such as myocardial ischemia from atherosclerosis, hypertension and myocardial cell dysfunction secondary to chronic hyperglycemia (Tripathi and Srivastava, 2006; Fowler, 2008). Low density lipoprotein particles found in type 2 diabetes are more atherogenic and are more easily glycated and susceptible to oxidation (Lyons and Jenkins, 1997; Durrington, 1999; Goldberg, 2001; Beauchamp *et al*., 2004).

2.2.2.5 Hypertension

Hypertension is up to twice as common in people with diabetes as in the general population. The central pathological mechanism in macrovascular disease is the process of atherosclerosis, which leads to narrowing of arterial walls throughout the body. Atherosclerosis is thought to result from chronic inflammation and injury to the arterial wall in the peripheral or coronary vascular system (Libby, 2001). In response to endothelial injury and inflammation, oxidized lipids from low density lipoprotein particles accumulate in the endothelial wall of arteries. This results in the loss of elastic tissue from the walls of the medium and large arteries, which consequently become rigid. When elastic tissue is lost the arteries become increasingly less able to absorb the pressure wave, which is pumped into the circulation with every heart beat, the pressure within the system therefore rises and the blood pressure goes up (Cooper *et al*., 2000; Libby, 2001). High blood pressure in diabetes appears to hasten the slide to kidney failure; it accelerates the process of atherosclerosis, and is also associated with an increased mortality from strokes and heart attacks (Cooper *et al*., 2000; Bastaki, 2005; Fowler, 2008).

2.2.2.6 Infections

Individuals with diabetes mellitus exhibit a greater frequency and severity of infection (Bertoni *et al*., 2001). The reasons for this include incompletely defined abnormalities in cell-mediated immunity and phagocyte function associated with hyperglycemia as well as diminished vascularization secondary to long-standing diabetes (Geerlings and Hoepelman, 1999; Mazade and Edwards, 2001). Many common infections are more frequent and severe in the diabetic population, whereas several rare infections are seen almost exclusively in the diabetic

population. These include rhinocerebral mucormycosis and malignant otitis externa, which is usually secondary to *Pseudomonas aeruginosa* infection in the soft tissue surrounding the external auditory canal (Lee *et al*., 1999; Ferguson, 2000; Roden *et al*., 2005; Cockram and Lee, 2010). Pneumonia, urinary tract infection, and skin and soft tissue infections are all more common in the diabetic patients. The most common organisms causing infections in diabetic patients are *Staphylococcus aureus, Escherichia coli, Streptococcus pneumoniae* and *Mycobacterium tuberculosis* (Laupland *et al*., 2004). Diabetic patients have an increased rate of colonization of *S. aureus* in skin folds and nares and also have a greater risk of postoperative wound infections (Tripathi and Srivastava, 2006).

2.3 Diagnosis of diabetes mellitus

The requirements for diagnostic confirmation for a person presenting with severe symptoms and gross hyperglycaemia differ from those for the asymptomatic person with blood glucose values found to be just above the diagnostic cut–off value (WHO, 1999). The clinical diagnosis of diabetes is often prompted by symptoms such as increased thirst and urine volume, recurrent infections, unexplained weight loss and in severe cases, drowsiness and coma. A single blood glucose estimation in excess of the diagnostic values indicated, establishes the diagnosis in such cases. An oral glucose tolerance test (OGTT) to establish diagnostic status need only be considered if casual blood glucose values lie in the uncertain range and fasting blood glucose levels are below those which establish the diagnosis of diabetes (Alberti, 2010). If an OGTT is performed, it is sufficient to measure the blood glucose values while fasting and at 2 hours after a

75 g oral glucose load. For children the oral glucose load is related to body weight: 1.75 g per kg. The diagnostic criteria in children are the same as for adults (Mahler and Alder, 1999; WHO, 1999).

The diagnosis of diabetes in an asymptomatic subject should never be made on the basis of a single abnormal blood glucose value. For the asymptomatic person, at least one additional plasma/blood glucose test result with a value in the diabetic range is essential, either fasting, from a random (casual) sample or from the oral glucose tolerance test (OGTT). If such samples fail to confirm the diagnosis of diabetes mellitus, surveillance with periodic re–testing until the diagnostic situation becomes clear is advised (WHO, 1999; Tripathi and Srivastava, 2006; Alberti, 2010).

The criteria for diagnosis of diabetes mellitus are either of the following: Symptoms of diabetes plus random blood glucose concentrations greater than 11.1 mmol/l (greater than 200 mg/dl), fasting plasma glucose greater than 7.0 mmol/l (greater than 126 mg/dl) (6.1 mmol l–1 (110 mg dl–1) for whole blood) or two-hour plasma glucose greater than 11.1 mmol/l (greater than 200 mg/dl) during an oral glucose tolerance test. Fasting is defined as no caloric intake for at least 8 hours (WHO, 1985; Expert Committee on the Diagnosis and Classification of Diabetes Mellitus, 1997; Expert Committee on the Diagnosis and Classification of Diabetes Mellitus, 2003; American Diabetes Association, 2010).

Though there is strong correlation between elevations in plasma glucose and glycated hemoglobin, the relationship between fasting plasma glucose and hemoglobin in individuals with normal glucose tolerance or mild glucose intolerance is less clear and the test is not universally standardized or available (Tripathi and Srivastava, 2006; American Diabetes Association, 2010).

2.4 Classification of diabetes mellitus

There are two major forms of diabetes: Type 1 and Type 2 diabetes mellitus. Type 1A diabetes mellitus is primarily due to autoimmune-mediated destruction of pancreatic β cell islets resulting in absolute insulin deficiency (Alberti, 2010). Type 1B diabetes mellitus is also characterized by insulin deficiency and a tendency to develop ketosis; however, individuals with type 1B diabetes mellitus lack the immunologic marker indicative of an autoimmune destructive process of β cells. Type 2 diabetes is characterized by insulin resistance and/or abnormal insulin secretion and increased glucose production. Distinct genetic and metabolic defects in insulin secretion/action give rise to the common phenotype of hyperglycemia (Kuzuya and Matsuda, 1997; Tripathi and Srivastava, 2006).

2.4.1 Type 1 diabetes

Type 1 diabetes represents approximately 10% of all cases of diabetes and develops secondary to autoimmune destruction of the insulin-producing β-cells of the pancreas (Mathis *et al.*, 2001). Due to the pathophysiology, insulin therapy is indicated at the onset of this disease. Type 1 diabetes represents a heterogenous and polygenic disorder, with a number of non-HLA loci

contributing to disease susceptibility (Concannon *et al*., 2009). There is yet no identified agent substantially capable of preventing this type of disease.

The WHO and the American Diabetics Association have proposed that type 1 diabetes can be divided into autoimmune/immune-mediated diabetes (Type 1A) and idiopathic diabetes with β-cell obstruction (Type 1B). This type of diabetes mellitus requires exogenous insulin to prevent diabetic ketoacidosis **(**Tripathi and Srivastava, 2006). Markers of the immune destruction of the β-cell include islet cell autoantibodies, autoantibodies to insulin, autoantibodies to glutamic acid decarboxylase (GAD65), and autoantibodies to the tyrosine phosphatases IA-2 and IA-2β. One and usually more of these autoantibodies are present in 85–90% of individuals when fasting hyperglycemia is initially detected (American Diabetes Association, 2010). Immune mediated diabetes commonly occurs in childhood and adolescence, but it can occur at any age, even in the 8^{th} and 9^{th} decades of life (Tuomi *et al*., 1993; American Diabetes Association, 2010).

A strong association has also been drawn between type 1 diabetes and human leukocyte antigen (HLA), with linkage to the DQA and DQB genes, and it is influenced by the DRB genes. These HLA-DR/DQ alleles can be either predisposing or protective (Redondo *et al.*, 2001; Concannon *et al*., 2009). In this form of diabetes, the rate of β-cell destruction is quite variable, being rapid mainly in infants and children and slow mainly in adults. Some patients, particularly children and adolescents, may present with ketoacidosis as the first manifestation of the disease. Others have modest fasting hyperglycemia that can rapidly change to severe hyperglycemia and/or ketoacidosis in the presence of infection or other stress. Still others, particularly adults, may

retain residual β-cell function sufficient to prevent ketoacidosis for many years; such individuals eventually become dependent on insulin for survival and are at risk for ketoacidosis. At this latter stage of the disease, there is little or no insulin secretion, as manifested by low or undetectable levels of plasma C-peptide (Pearson *et al*., 2003; Alberti, 2010; American Diabetes Association, 2010).

Autoimmune destruction of β-cells has multiple genetic predispositions and is also related to environmental factors that are still poorly defined. Although patients are rarely obese when they present with this type of diabetes, the presence of obesity is not incompatible with the diagnosis (American Diabetes Association, 2010).

2.4.2 Type 2 diabetes

This form of diabetes accounts for 90–95% of those with diabetes. Type 2 diabetes results from a combination of defects in insulin secretion and insulin action, either of which may predominate (WHO, 1999). At least initially, and often throughout their lifetime, these individuals with type 2 diabetes do not need insulin treatment to survive, but may require it for the control of blood glucose levels if this is not achieved with diet alone or with oral hypoglycemic agents. Type 2 diabetes is a progressive disorder, which is associated with diminishing pancreatic function over time. Recognition of the phase is important in the clinical management of the disorder because depending on the stage, effective control may require lifestyle modification, oral agent therapy, oral agents combined with insulin, or insulin alone (Weyer *et al*., 1999, 2001; Buchanan 2003). Insulin resistance, defined as a clinical state in which a normal or elevated insulin level produces

an inadequate biological response, is considered to be a hallmark for the presence of metabolic syndrome and type 2 diabetes (Hunter and Garvey, 1998). The cellular mechanisms that contribute to insulin resistance are not fully understood (Alberti, 2010).

The presence of insulin resistance in an individual must be compensated by hyperinsulinemia to maintain normal glucose tolerance (Kahn, 2000; Buchanan, 2003). It has also been observed that in those individuals who develop diabetes, a progressive loss of the insulin secretory capacity of β-cells appears to begin years before the clinical diagnosis of diabetes (Weyer *et al*., 1999, 2001; Buchanan, 2003). The pancreatic dysfunction fails to compensate for the insulin resistance and results in a state of relative "insulin deficiency" leading to hyperglycemia. It is at this stage that impaired glucose tolerance and impaired fasting glucose may be present (Cefalu, 2000). With worsening islet dysfunction and the inability to compensate fully for the degree of insulin resistance, clinically overt type 2 diabetes develops (Weyer *et al*., 1999, 2001; Buchanan, 2003).

Although the specific etiologies are not known, autoimmune destruction of β-cells does not occur in type 2 diabetes. The risk of developing this form of diabetes increases with age, obesity and lack of physical activity. It occurs more frequently in women with prior gestational diabetes mellitus and in individuals with hypertension or dyslipidemia, and its frequency varies in different racial or ethnic subgroups (Reaven, 1988; Defronzo, 1992; Haffner, 1996; Liese *et al.*, 1998; Isomaa *et al.*, 2001). It is often associated with a strong genetic predisposition, more so than is the autoimmune form of type 1 diabetes. However, the genetics of this form of diabetes

are complex and not clearly defined (American Diabetes Association, 2010). Patients who are not obese by traditional weight criteria may have an increased percentage of body fat distributed predominantly in the abdominal region **(**Tripathi and Srivastava, 2006).

Ketoacidosis in this type of diabetes usually arises in association with the stress of another illness such as infection as opposed to spontaneous ketoacidosis in type 1 diabetes. This form of diabetes may go undiagnosed for many years because the hyperglycemia develops gradually and at earlier stages is often not severe enough for the patient to notice any of the classic symptoms of diabetes. Nevertheless, such patients are at increased risk of developing macrovascular and microvascular complications (Weyer *et al.*, 1999, 2001; Buchanan, 2003; Tripathi and Srivastava, 2006). Whereas patients with this form of diabetes may have insulin levels that appear normal or elevated, insulin secretion is defective in these patients and insufficient to compensate for insulin resistance (Tripathi and Srivastava, 2006).

2.5 Diabetes in Kenya

Epidemiological surveys conducted by the Nairobi-based Diabetic Management and Information Center (DMI) give the estimated prevalence of diabetes mellitus in Kenya at 3% in 2003 and above 6% in 2007 (IDF, 2007). In some rural parts of the country such as Nyeri in central Kenya and Kilifi in the Coast province the prevalence is as high as 11.6% and above 20% among the richer families in the major urban centers (Chege, 2010).

The Ministry of Health estimates the prevalence of diabetes to be around 10% (3.5 million people (Jalang'o, 2006). The cause of much human suffering, diabetes also places a considerable economic burden on individuals, families and healthcare systems. The number of people with obesity-related type 2 diabetes appears to be rising sharply as the sedentary behaviours and high-fat, high-sugar foods that are typical of expanding urban poverty replace the constant physical activity and vegetable-based diet that characterize the rural lifestyle (McFerran, 2008).

2.6 Management of Diabetes

Generally, current therapeutic strategies for type 2 diabetes are limited and involve insulin and four main classes of oral antidiabetic agents that stimulate pancreatic insulin secretion, reduce hepatic glucose production, delay digestion and absorption of intestinal carbohydrate or improve insulin action (Gough and Narendran, 2010). The hyperglycemia observed in diabetes mellitus is the result of a mismatch between the quantity of insulin necessary to regulate metabolic processes and the amount of insulin being secreted by the β-cells. Insulin replacement therapy is the mainstay for patients with type 1 diabetes mellitus while diet and lifestyle modifications are the basis for the treatment and management of type 2 diabetes mellitus in its initial stages. Insulin is also important in type 2 diabetes mellitus when blood glucose levels cannot be controlled by diet, weight loss, exercise and oral medications (Bastaki, 2005; Tripathi and Srivastava, 2006; Gough and Narendran, 2010).

Insulin was discovered by Banting and Best in 1922 completely revolutionizing the treatment of diabetes mellitus. Progress has been made, in recent years, in the production, formulation and delivery of insulin preparations, as well as the development of insulin treatment regimens which maintain long-term-normoglycaemia with a low risk of hypoglycemia. Insulin is the most potent glucose-lowering agent, with hypoglycemia being the only major dose-limiting factor. Insulin has progressively more side effects as the dose is increased and may be administered intravenously or intramuscularly. However for long-term treatment, subcutaneous route is preferred (Bastaki, 2005).

Insulin significantly reduces glucose concentrations by suppressing hepatic glucose production, increasing postprandial glucose utilization and improving the abnormal lipoprotein that is characteristic of insulin resistance. Insulin therapy may also decrease or eliminate the effects of glucose toxicity by reducing hyperglycemia to improve insulin sensitivity and β-cell secretory function (Soeborg *et al*., 2009). With the advent of recombinant DNA technology, more adaptable forms of insulin analogues have been designed. The subsequent availability of rapid-acting (insulin lispro, insulin aspart) and long acting (insulin glargine and detemir insulin) insulin analogues for meal and basal requirements offer both individual and collective advantages (Yadav and Parakh, 2006; Monami *et al*., 2008). The developments towards insulin delivery led to external continuous subcutaneous insulin infusion pumps, capable of achieving excellent metabolic control and reduced risk of hypoglycemia (Tripathi and Srivastava, 2006).

Oral hypoglycaemic agents are important in the treatment of type 2 diabetes mellitus where there are residual functioning pancreatic β-cells. However, owing to the progressive nature of the disease, oral antidiabetic agents even when used intensively are often unable to control the hyperglycemia (Wallace and Matthews, 2003; Krentz and Bailey, 2005a). Consequently, within 4-5 years, therapy can no longer compensate for β-cell failure. Oral hypoglycaemic agents include sulphonylureas, biguanides, α-glucosidase inhibitors, thiazolidinediones and more recently meglitinide analogues (Krentz and Bailey, 2005a; Krentz and Bailey, 2005b; Krentz *et al*., 2008; Bailey, 2009).

Sulphonylureas act directly on the islet β- cells to close ATP-sensitive K+ channels, which stimulate insulin secretion (Ashcroft and Gribble, 1999; Gribble and Reimann, 2002). The efficacy of sulphonylureas depends on the presence of a functional pancreas (Ball *et al*., 2000). Sulphonylureas are associated with hypoglycemia with the risk being higher in the elderly and patients with renal insufficiency. The first generation of sulfonylureas included tolbutamide, acetohexamide, tolazamide, and chlorpropamide. A second generation of more effective sulphonylureas has been developed and includes glibenclamide, glipizide, gliclazide, and glimepiride (Ball *et al*., 2000; Bastaki, 2005; Tripathi and Srivastava, 2006).

Metformin is the most commonly used biguanide. Its mechanisim of action is not fully understood although it is antihyperglycemic and not hypoglycemic. When used alone or in combination with a sulfonylurea, metformin improves glycemic control and lipid concentrations

in patients who are nonresponsive to sulphonylureas (Grant, 2003; Bailey, 2008**)**. Metformin improves insulin resistance in the liver, skeletal muscle and adipose tissue (Zhou *et al*., 2001). It also reduces hepatic glucose output. Its efficacy in reducing fasting plasma glucose and postprandial glucose concentrations is similar to that of sulphonylureas but in contrast it does not cause weight gain or hypoglycaemia (Cusi and DeFronzo, 1998; Bastaki, 2005; Tripathi and Srivastava, 2006).

Rosiglitazone and pioglitazone are the two thiazolidinediones currently in use. Thiazolidinediones act by lowering insulin resistance in peripheral tissue, but an effect to lower glucose production by the liver has also been reported (Staels and Fruchart, 2005; Semple *et al*., 2006). Thiazolidinediones increase glucose transport into muscle and adipose tissue by enhancing the synthesis and translocation of specific forms of the glucose transporter proteins. The thiazolidinediones can also activate genes that regulate free fatty-acid metabolism in peripheral tissue, thus lowering triglycerides and non-esterified fatty acid levels and inducing differentiation of adipocytes. Their blood glucose-lowering activity is dependent on the presence of at least normal circulating levels of insulin and efficacy is greater in combination with insulin therapy or an insulin releaser (Kahn *et al*., 2006). Thiazolidinediones are especially effective in combination with insulin to reduce the high insulin dosage and improving glycemic control in type 2 diabetes. They are also used effectively in combination with other classes of antidiabetic agents (Nesto *et al*., 2003; Bastaki, 2005; Tripathi and Srivastava, 2006).

The meglitinide analogues are a new class of drugs developed from the meglitinide portion of sulphonylureas (Garrino *et al*., 1985). They work by improving early-phase insulin secretion and examples are repaglinide and nateglinide (Blickle, 2006). Another meglitinide known as mitiglinide though not currently approved in the United States and Europe, recently received approval for use in Japan (Phillippe and Wargo, 2013). Repaglinide is derived from the non-sulphonylurea moiety of Glibenclamide whereas nateglinide is derived from the amino acid D phenylalanine. The meglitinides are rapid-acting insulin secretagogues that have a fast onset and short duration of action resulting in more physiological secretion of insulin from the β-cell without causing continued elevation of insulin in the post absorptive phase, thus reducing glycaemia without increasing the risk of hypoglycaemia. The mechanism of action of meglitinides is glucose-dependent (Dornhorst, 2001; Davies, 2002; Blickle, 2006).

Alpha-Glucosidase inhibitors competitively inhibit α-glucosidases that are associated with the brush border membrane of the small intestine and are responsible for the digestion of complex polysaccharides and sucrose. This slows carbohydrates digestion and lowers post-prandial hyperglycemia. They can be used as monotherapy or in combination with other oral antidiabetic drugs. The three α-glucosidase inhibitors that have been developed are acarbose, miglitol, and voglibose (Lebovitz, 1998; Holman *et al*., 1999; Bailey and Day, 2009).

2.7 Alternative medicine for diabetes

The field of herbal medicines research has been gaining significant importance in the last few decades and the demand to use natural products in the treatment of diabetes is increasing

worldwide. Presently, over 400 traditional plant treatments for diabetes have been reported, although only a small number of these have received scientific and medical evaluation to assess their efficacy (Mukherjee, 1981; Marles and Farnsworth, 1995; Rai, 1995; Dropkin, 2010; Kolling *et al.*, 2010; Gunjan *et al.*, 2011). Metformin is an effective oral glucose-lowering agent which was developed based on the use of *Galega officinalis* to treat diabetes. *Galega officinalis* is rich in guanidine, the hypoglycemic component (Gunjan *et al.*, 2011).

The hypoglycemic effect of some herbal extracts has been confirmed in human and animal models of type 2 diabetes (Jarald *et al*., 2008; Kavishankar *et al*., 2011; Gupta and De, 2012; Pandeya *et al*., 2013; Preethi, 2013). The World Health Organization Expert Committee on diabetes recommended that traditional medicinal herbs be further investigated (Bailey and Day, 1989). The major obstacle in the integration of herbal medicine into modern medical practices is the lack of scientific and clinical data proving their efficacy and safety. There is a need for clinical research in herbal drugs using appropriate bioassays for biological standardization, pharmacological and toxicological evaluation and developing various animal models for toxicity and safety evaluation. It is also important to establish the active component/s from these plant extracts (Gunjan *et al*., 2011).

A scientific investigation of traditional herbal remedies for diabetes may provide valuable leads for the development of alternative drugs and strategies. Alternatives are clearly needed for better

management of diabetes because of high cost and poor availability of current therapies for many rural populations, particularly in developing countries.

A study by Dropkin (2010) found that a number of herbal and alternative medicine options are available for diabetes care in Mombasa. However, it is difficult to comment on the quality of care provided because studies evaluating the efficacy of these treatments, which can differ greatly between providers, are extremely limited. The author reports that many patients get frustrated with the slow progress they are making with conventional care and look for a quick fix through alternative medicine which was also noted by Kolling *et al* (2010).

In spite of the presence of known antidiabetic medicines in the pharmaceutical market, remedies from medicinal plants are used with success to treat this disease (Bhattaram *et al.*, 2002). Many traditional plant treatments for diabetes are used throughout the world. Plant drugs (Bailey and Day, 1989) and herbal formulations (Mitra *et al.*, 1996; Bhattacharya *et al.*, 1997; Annapurna *et al.*, 2001) are frequently considered to be less toxic and have fewer side effects compared to synthetic ones. Based on the WHO recommendations, hypoglycemic agents of plant origin used in traditional medicine are important (WHO Expert Committee on Diabetes Mellitus, 1980). The attributed antihyperglycemic effects of these plants are due to their ability to restore the function of pancreatic tissues by causing an increase in insulin output or inhibit the intestinal absorption of glucose or to the facilitation of metabolites in insulin dependent processes. Achieving

glycemic targets in type 2 diabetes remains a great challenge during clinical care (Cook *et al.*, 2001).

In general, there is very little biological knowledge on the specific modes of action in the treatment of diabetes, but most of the plants have been found to contain substances like glycosides, alkaloids, terpenoids and flavonoids that are frequently implicated as having antidiabetic effects (Loew and Kaszkin, 2002). *Ovariodendron anisatum, Schkuhria pinnata, Mangifera indica, Xerophyta spekei, Lonchocarpus eriocalyx* have been documented as being used for treatment of diabetes in Mbeere and Embu districts of Kenya (Kareru *et al.*, 2007).

2.7.1 *Zanthoxylum chalybeum*

Zanthoxylum comprises about 549 species distributed worldwide mainly in tropical and temperate regions (Global Biodiversity Information Facility, 2010). This genus includes trees and shrubs, usually dioecious in the family Rutaceae. The trees have leafy crown, with few branches and reach up to 20 meters. The species of this genus are characterized by the presence of recurved spines on its trunk and branches. The leaves are varied, may be alternate or opposite, simple or compound (Kumar and Paridhavi, 2012; Patino *et al.*, 2012).

Zanthoxylum chalybeum Engl. family: Rutaceae is a deciduous spiny shrub or tree up to 12 m, crown rounded but open. Bark pale grey; smooth dark with scales and prickles. The trunk has characteristic large, conical, woody knobs with sharp prickles. The branches also bear scattered thorns with conspicuous dark scales. Leaves are compound, usually 3-5 pairs of shiny leaflets

plus a terminal leaflet; leaflets are oblong to elliptic or lanceolate, 2.5-7 x 1-2.5 cm, with a strong citrus smell when crushed. Flowers are sweet scented, yellowish-green, in racemes or panicles 5-10 cm long, produced immediately below the leaves at the base of the new branchlets. Fruits are spherical, about 5 mm in diameter, reddish-brown, splitting to allow the shiny black seeds to partly protrude (Pattino *et al*., 2012).

Zanthoxylum chalybeum grows in medium to low altitudes in dry woodland or grassland, often on termite mounds. It is native to Burundi, Democratic Republic of Congo, Ethiopia, Kenya, Lesotho, Malawi, Mozambique, Namibia, Rwanda, Somalia, South Africa, Swaziland, Tanzania, Uganda, Zambia and Zimbabwe. It grows at an altitude of up to 1 600 m and a mean annual rainfall of 750-1 500 mm. Bark extracts are said to cure malaria (Beentje, 1994; Katende, 1995).

Zanthoxylum chalybeum stem bark is commonly used in parts of Asia and East Africa to treat diabetes. Decoctions are used to treat diabetes mellitus related symptoms in Kenya, Tanzania and Asia (Moshi and Mbwambo, 2002; Keter and Mutiso, 2012). *Z. chalybeum* Engl. Roots are used to treat diabetes and related symptoms in Tanzania (Moshi and Mbwambo, 2002). In Kenya, *Z. chalybeum* stem bark decoction is used to treat diabetes. Based on the available literature, the hypoglycaemic activity of *Z. chalybeum* has not been validated experimentally in the in vivo and in vitro diabetic models, clinical and chemical studies (Keter and Mutiso, 2012).

The plant is also used for treatment of malaria, sickle cell disease, measles, tuberculosis, skin infections and coughs (Olila *et al*., 2002; Tabuti, 2008; Rukunga *et al*., 2009). In Tanzania, *Z. chalybeum* root bark is used to treat oesophageal candidiasis. Root bark is powdered and added to tea then taken orally (Runyoro *et al*., 2006). *Z. chalybeum* has also been shown to possess antitrypanosomal activity IC_{50} values between 1 and 16µg/ml (Rukunga *et al*., 2009).

Other species in the genus *Zanthoxylum* also possess antidiabetic activity. For example, various parts of *Z. zanthoxyloides* including the roots, bark and leaves have been used for medicinal purposes, including the treatment of diabetes mellitus. Significantly ($P < 0.05$) lower blood glucose was observed in the treated animals in comparison to non-treated groups (Aloke *et al*., 2012). *Zanthoxylum armatum* is used in Nepal to treat diabetes (Singh and Singh, 2011). In India *Z. nitidum* is used traditionally to treat diabetes (Kumar and Paridhavi, 2012).

Medicinal properties of this genus have been attributed to the presence of secondary metabolites like alkaloids, sterols, flavonoids, aliphatic and aromatic amides, lignins, coumarins, sterols, carbohydrate residues (Aloke *et al*., 2012).

2.7.2 *Ficus sycomorus*

Ficus sycomorus Linn belongs to the family Moraceae, comprising about 40 genera and over 1,400 species of trees, shrubs, vine and herbs, often with milky latex juices. They are usually found near streams in the savannah area. *F. sycomorus* is a tree that grows up to 20 m with widely spreading branches and a massive crown. Sheep and cattle eat its foliage (El-Sayed *et al*.,

2010; Sarg *et al*., 2011; Aduom *et al*., 2012). *Ficus* species are used as food and for treatment of various diseases such as ulcers, vomiting, vaginal complaints, fever, diabetes, inflammation and liver disease, malaria, cancers, hypertension, stomach problems, aphrodisiacs, analgesics, antimicrobials and antihelmintics (El-Sayed *et al*., 2010; Sarg *et al*., 2011).

Ficus sycomorus is used in Nigeria, Niger, Mali, South Africa, Guinea, Kenya, Tanzania, Somalia, Ethiopia and Ivory Coast as extracts of fruits, leaves, root and stem barks to treat various ailments such as cough, diarrhea, skin infections, stomach disorders, liver disease, epilepsy, tuberculosis, lactation disorders, helminthiasis, infertility and sterility (Igbokwe *et al*., 2010; Aduom *et al*., 2012). The plant has also been reported to be a potent antimicrobial agent against ciprofloxacin resistant *Salmonella typhi*. The Hausa and Fulani tribes of northern Nigeria use the stem-bark of *F. sycomorus* to treat diabetes mellitus, fungal diseases, jaundice and dysentery (Hassan *et al*., 2007; Aduom *et al*., 2012). In Palestine, the milky sap from *F. sycomorus* stem bark is used for treating skin diseases while a decoction of the stem bark is used for problems of the digestive system. It is also used as seasoning, leaves are dried and added to cake as a condiment, eaten raw or cooked as soup. Dry branches of the species are collected for use as fuel (Auda, 2012).

Aduom *et al* (2012) established the LD_{50} of the methanol extract of *F. sycomorus* as 1500mg/kg. In this study, the methanol stem bark extract of *F. sycomorus* at a dose of 250mg/kg body weight, elicited significant hypoglycemic activity in diabetic rats, $P<0.05$. With glucose levels

reverting back to normal control levels, compared with untreated diabetic animals. The hypoglycemic effect of the methanolic stem-bark extract of *F. sycomorus* was not dose-dependent.

Njagi *et al* (2012) determined the effect of aqueous stem bark extract on blood glucose on alloxan induced diabetic rats. The percent reductions of blood glucose levels in mice by the aqueous stem bark extract of *F. sycomorus* at the three dose levels (50 mg/kg body weight, 100mg/kg body weight and 150 mg/kg body weight) during the 1st hour were 30%, 28 % and 49%, respectively. Results indicated that all three dose levels of the extract lowered blood glucose levels as effectively as insulin 3 hours after treatment (Njagi *et al*., 2012).

The medicinal properties of this genus have been attributed to presence of flavonoids, tannins, coumarins, terpenes, phytosterols, alkaloids and their glycosides (El-Sayed *et al*., 2010; Sarg *et al*., 2011). The phytochemical analysis of the methanolic stem-bark extract of *F. sycomorus* revealed the presence of flavonoids, saponins, alkaloids, reducing sugars and glycosides. Some flavonoids and glycosides have been found to stimulate β-cells regeneration, increase insulin secretion or possess insulin-like effects (Aduom *et al*., 2012). Antioxidant studies of the chemical constituents of *F. sycomorus* leaves revealed that quercetin, gallic acid, rutin, isoquercitrin, quercetin 3, 7-*O*-α-L-dirhamnoside are the major constituents of the plant and represent the antioxidant ingredients. Thus, the presence of these free radical scavengers in *F. sycomorus* might be relevant in relation to this plants various biological properties and medicinal

uses (El-Sayed *et al*., 2010). Hassan *et al* (2007) reports that the aqueous ethanolic stem bark extract contains tannins, saponins, reducing sugars, flavon aglycones, anthraquinone glycosides, flavonoid glycosides and condensed tannins.

Other species in the genus *Ficus* have also been studied for their antidiabetic activity (Khan *et al*., 2012). The, alcoholic extract of *F. bengalensis* stem bark at a dose of 25, 50 & 75mg/day/100g body weight lowered the blood sugar level 47 to 70%, and also restored the normal levels of serum urea, cholesterol and total protein of alloxan diabetic albino rats (Gupta *et al*., 2008). The Yoruba-speaking people of Western Nigeria often employ decoctions and infusions of *F. exasperata* leaves traditionally for the treatment of various human diseases, including diabetes mellitus (Adewole *et al*., 2011). Continuous treatment of STZ-treated spontaneously hypertensive rats (SHR) and obese Zucker diabetic rats with ethanolic extract of *F. exasperata* (FEE) for a period of 4 weeks caused significant decrease ($p<0.05$) in blood glucose levels of the FEE treated diabetic rats (Adewole *et al*., 2011). *F. retusa* L. "variegata", alcoholic extract (400 mg/kg) was found to reduce blood glucose levels of diabetic rats significantly as compared to the diabetic group (Sarg *et al*., 2011).

F. bengalensis is well known in the treatment of diabetes (Dhungana *et al*., 2013). Studies have reported that *F. bengalensis, F. carica* and *F. glomerata* are effective in the treatment of diabetes. The ethanol extract of leaves of *F.glomerata* has significant antihyperglycemic effect in experimental albino rat model of diabetes mellitus. The fruits of *F. glomerata*, locally known as

Gular have been used since ancient times in the ethno-medicine as a remedy of diabetes mellitus. The aqueous extract of *F. bengalensis* at a dose of 500mg/kg/day exhibited significant antidiabetic and amelioferative activity as evidenced by histological studies in normal and *F. bengalensis* treated streptozotocin induced diabetic rats. *Ficus exasperate* Vahl and *F. arnottiana* Miq. are also reported to have antidiabetic activity (Khan *et al*., 2012; Dhungana *et al*., 2013).

The antidiabetic activity of various *Ficus* spp. is postulated to be due to the presence various chemical compounds including, alkaloids, flavonoids, saponins, tannins, glycosides, gallic and reducing sugars. Additionally, elements including K, Ca, Cr, Mn, Fe, Cu and Zn which are responsible for initiating insulin function have been shown to be present, but the levels differ with the plant part and species (Khan *et al*., 2012).

Clinical research suggests that diabetes causes the disruption of mineral trace elements in body. These trace elements play an important role in the production of secondary metabolites which are responsible for pharmacological actions of medicinal plants. But the exact mechanism of these active metabolites is yet to be established. The elements potassium, Calcium, Manganese, Copper and Zinc have been reported to be responsible for the secretion of insulin from the beta cells of pancreas (Khan *et al.*, 2012). There is a need to study further pharmacological activity, toxicological effects and the exact mechanism of the extract in the search for ideal alternative drugs, especially in underdeveloped countries. The elements present in *Ficus* species have an

important role in the treatment of diabetes. Results of previous work have shown variation in elemental composition of medicinal plants from region to region, thus there is a need to vouch for more research on medicinal plants to integrate their medicinal values in the advance system of medicine preparation (Khan *et al*., 2012).

The aqueous extract of *F. sycomorus* stem bark contained pharmacologically active substances such as gallic, tannins, saponins, reducing sugars, alkaloids and flavones aglycones and caused no haematological, hepatic and renal toxicities (Igbokwe *et al*., 2010).

2.7.3 *Ximenia americana*

The genus *Ximenia* belongs to the family Olacaceae and comprises about 8 species: *X. roiigi*, *X. aegyptiaca*, *X. parviflora*, *X. coriaceae*, *X. aculeata*, *X. caffra*, *X. americana* and *X. aegyptiaca* (Monte *et al*., 2012). *X. americana* Linn. is the most common. It is commonly known as false sandal wood, Wild Plum, tallow wood, Sour Plum, Yellow Plum or Sea Lemon. It is found mainly in tropical regions (Africa, India, New Zealand, Central America and South America), especially Africa and Brazil. The plant is a small tree reaching a height of up to 6 metres, with gray or reddish bark, with leaves small, simple, alternate of bright green color and with a strong smell of almonds. The flowers are white or yellowish-white, curved and aromatic. Fruit are yellow-orange, aromatic, measuring 1.5 to 2.0 cm in diameter, surrounding a single seed and have a pleasant plum-like flavor (Okigbo *et al*., 2009; Feyssa *et al*., 2012; Monte *et al*., 2012; Shantha *et al*., 2012).

It is a plant of diverse habitats in semi-arid bushland, in many types of dry woodland, sandy open woodland and dry hilly areas and coastal bushlands. It is frequently found on coastal dunes, along water courses and on stony slopes. It occurs at altitudes up to 2000m above sea level and where rainfall exceeds 500mm per year and temperatures of 14 -30 ^{0} C. It grows on many soil types; however, often on poor and dry types (Feyssa *et al*., 2012; Shantha *et al*., 2012).

In Asia, the young leaves are consumed as a vegetable, however, the leaves also contain cyanide and need to be thoroughly cooked, and should not be eaten in large amounts (Monte *et al*., 2012). In Brazil, a tea obtained from *X. americana* stem bark has been used in popular medicine as cicatrizing, astringent and as an agent against excessive menstruation. As a powder, it treats stomach ulcers and the seeds are purgative (Monte *et al*., 2012). In the Indian system of medicine, the various plants parts like leaves, roots, bark, roots and fruits are used for the treatment of diabetes, mouth ulcers, malaria, cancer, diarrhoea fever and inflammation (Siddaiah *et al*., 2011; Shantha *et al*., 2012). In Mali, *X. americana* roots and leaves are used to treat throat infection, malaria, wounds and dysmenorrhea. In Nigeria the tree has been used against malaria, leprotic ulcers and skin diseases, schistosomiasis, fever, diarrhoea, ringworm, river blindness and tooth ache (Okigbo *et al*., 2009; Le *et al*., 2012; Shantha *et al*., 2012). In tropical West Africa, the root has been used medically for febrile headache. An infusion or a decoction of the root is drunk as medicine for venereal disease. In Tanzania, the root is used as a febrifuge and diarrhoea remedy. In Zimbabwe, a decoction of the leafy twigs is given for febrile colds and cough and as laxative (Okigbo *et al*., 2009; Shantha *et al*., 2012).

Investigations have shown that the constituents of *X. americana* posses several biological activities such as, antimicrobial, antifungal, antidiabetic, anticancer, antineoplastic, antitrypanosomal, antirheumatic, antioxidant, analgesic, molluscicide, pesticidal, also having hepatic and hematological effects (Siddaiah *et al*., 2011; Monte *et al*., 2012). Oral administration of the methanolic extract of *X. americana* leaves (200, 400 and 600mg/kg body weight) for seven days resulted in a significant reduction in blood glucose level in alloxan induced hyperglycemic rats. The effect was compared to that of 0.5gm/kg (i.p) Glibenclamide (Siddaiah *et al*., 2011). From phytochemical analysis of crude *X. americana* aqueous, methanolic, ethanolic, butanolic and chloroform extracts from different parts (leaves, root, stem and stem bark) the secondary metabolites contained were saponins, glycosides, flavonoids, tannins, phenolics, alkaloids, quinones and terpenoids types. In addition, the plant is potentially rich in fatty acids and glycerides and the seeds contain derivatives of cyanide (Siddaiah *et al*., 2011; Monte *et al*., 2012). Flavonoids and tannins have been shown to have antidiabetic activity (Siddaiah *et al*., 2011).

Work on plants of the genus *Ximenia* is justified, particularly *X. americana* species, where systematic study relative to specific biological activity of their chemical constituents is not comprehensive (Monte *et al*., 2012). There is oral evidence indicating that the plant is effective in many disease conditions including diabetes mellitus but there are few documented scientific studies (Shantha *et al*., 2012).

2.8 Animal models for diabetes

Different animal models of type 1 and type 2 diabetes for have been used for screening for antidiabetic activity of novel drugs. These range from surgical models, genetic models, various animal strains that spontaneously develop diabetes, and chemical models of diabetes mellitus. The species of animal used is determined by several factors. Generally, smaller animals are more manageable and cheaper hence, rats and mice are the most commonly used.

One of the most commonly used methods for inducing diabetes is by damaging the pancreas by the administration of chemicals such as streptozotocin (STZ) and alloxan. These animal models mimic several characteristics of the human disease. Chemically induced models of diabetes mellitus enable for evaluation of blood glucose following treatment with a novel test drug. Results are compared to non-diabetic or diabetic animals treated with conventional antidiabetic drugs. A type 1 diabetic rat model has been developed using the Wistar rat by injecting adult rats with a single dose of streptozotocin at 45 mg/kg, intraperitoneally (Ramesh and Pugalendi, 2006; Gayathri and Kannabiran, 2008). The streptozotocin-induced Wistar rat develops complications associated with hyperglycemia, similar to the human diabetic situation. Thus this diabetic rat is a suitable model for the investigations into the pathology of diabetes mellitus and complications related to the disease as well as possible interventions (Ramesh and Pugalendi, 2006; Gayathri and Kannabiran, 2008; Deeds *et al*., 2011).

Rodents also show a substantial gender difference in STZ sensitivity. Male mice and rats tend to be more susceptible to STZ-induced diabetes. This decreased sensitivity experienced by females may be attributed to oestradiol's ability to protect pancreatic β-cells from apoptosis induced by oxidative stress (Deeds *et al*., 2011).

2.9 Streptozotocin

Streptozotocin (2-deoxy-2-(3-(methyl-3-nitrosoureido)-D-glucopyranose) is a broad spectrum antibiotic synthesized by *Streptomycetes achromogenes.* It is used clinically for the treatment of metastatic islet cell carcinoma of the pancreas. Experimentally, it has been used in different animal species to induce both type 1 and type 2 diabetes mellitus (Szkudelski, 2001; Deeds *et al*., 2011).

The frequently used single intravenous dose in adult rats to induce type 1 diabetes is 40-60 mg/kg body weight but higher doses are also used. STZ is also efficacious after intraperitoneal administration of a similar or higher dose, but single dose below 40 mg/kg body weight may be ineffective. STZ may also be given in multiple low doses (Szkudelski, 2001; Srinivasan and Ramarao, 2007; Deeds *et al*., 2011).

Streptozotocin action in β cells is characterized by alterations in blood insulin and glucose concentrations. Hyperglycemia and a drop in insulin are observed two hours after injection. This is followed six hours later by hypoglycemia with high levels of blood insulin. Finally,

hyperglycemia develops and blood insulin levels decrease. These changes in blood glucose and insulin concentrations reflect abnormalities in β-cell function. STZ impairs glucose oxidation and decreases insulin synthesis and secretion (Szkudelski, 2001).

STZ is taken up by the pancreatic β-cells *via* glucose transporter GLUT2. The main reason for the STZ-induced β -cell death is alkylation of DNA. The alkylating activity of STZ is related to its nitrosourea moiety, especially at the O6 position of guanine. Since STZ is a nitric oxide (NO) donor and NO was found to bring about the destruction of pancreatic islet cells, it was proposed that this molecule contributes to STZ-induced DNA damage (Morgan *et al*., 1994; Kröncke *et al*., 1995). However, the results of several experiments provide the evidence that NO is not the only molecule responsible for the cytotoxic effect of STZ. STZ was found to generate reactive oxygen species, which also contribute to DNA fragmentation and evoke other deleterious changes in the cells. The formation of superoxide anions results from both STZ action on mitochondria and increased activity of xanthine oxidase (Szkudelski, 2001).

It was demonstrated that STZ inhibits the Krebs cycle and substantially decreases oxygen consumption by mitochondria. These effects strongly limit mitochondrial adenosine triphosphate (ATP) production and cause depletion of this nucleotide in β-cells. Restriction of mitochondrial ATP generation is partially mediated by NO. Augmented ATP dephosphorylation increases the supply of substrate for xanthine oxidase (β-cells possess high activity of this enzyme) and enhances the production of uric acid – the final product of ATP degradation. Xanthine oxidase

then catalyses the reaction in which the superoxide anion is formed. As a result of superoxide anion generation hydrogen peroxide and hydroxyl radicals are formed. STZ-induced DNA damage activates poly (adenosine diphosphate) ADP ribosylation. This process leads to depletion of cellular Nicotinamide adenine dinucleotide (NAD^+), further reduction of the ATP content and subsequent inhibition of insulin synthesis and secretion. Streptozotocin causes alkylation or breakage of DNA strands and a consequent increase in the activity of poly-ADP-ribose synthetase, an enzyme depleting NAD in β-cells finally leading to energy deprivation and death of β-cells is reported (Srinivasan and Ramarao, 2007; Szkudelski, 2001).

The potent alkylating properties of STZ are the main cause of its toxicity. However, the synergistic action of both NO and reactive oxygen species may also contribute to DNA fragmentation and other deleterious changes caused by STZ (Szkudelski, 2001).

2.10 Glucose measurement

According to the recommendations of the American Diabetes Association, self monitoring blood glucose should be used in patients on intensive insulin therapy and may also be useful in patients using less frequent insulin injections, noninsulin therapies, or medical nutrition therapy alone (American Diabetes Association, 2010). Glucose monitoring is important when evaluating treatment regimens in diabetic patients as well as in the experimental set up when evaluating novel products for antidiabetic activity (Polsup *et al*., 2008). During the last five decades a significant improvement in glucose biosensor technology including point-of-care devices,

continuous glucose monitoring systems and noninvasive glucose monitoring systems has been made (Wang, 2008).

The glucose biosensors are divided into five classes based on the type of transducer used. These are; electrochemical, optical, thermometric, piezoelectric and magnetic (Newman and Turner, 1992). Majority of the current glucose biosensors are of the electrochemical type. They provide better sensitivity, reproducibility, are easy to maintain and low cost. Electrochemical sensors are subdivided into potentiometric, amperometric, or conductometric types (Habermuller *et al*., 2000; Pearson *et al*., 2000; Thevenot *et al*., 2001). Enzymatic amperometric glucose biosensors are the most common devices commercially available. In this study, an amperometric glucose meter was used. Amperometric sensors monitor currents generated when electrons are exchanged either directly or indirectly between a biological system and an electrode glucose oxidase is the standard enzyme for biosensors. It has a relatively higher selectivity for glucose (Wang, 2008; Yoo and Lee, 2010).

The glucose biosensor operates on the principle that the immobilized glucose oxidase catalyzes the oxidation of glucose by molecular oxygen producing gluconic acid and hydrogen peroxide.

$$\text{Glucose} + O_2 + 2H_2 \rightarrow \text{Gluconic acid} + H_2O_2$$

$$H_2O_2 \longrightarrow 2H^+ + O_2 + 2e$$

Hydrogen peroxide is oxidized at a catalytic, platinum anode. The electrode recognizes the number of electron transfers and this electron flow is proportional to the number of glucose molecules present in blood (Wang, 2008; Yoo and Lee, 2010).

2.11 Objectives

The study aimed at documenting the plants that are used to treat diabetes mellitus in Machakos County in eastern Kenya. Three most commonly employed plants, *Zanthoxylum chalybeum, Ximenia americana and Ficus sycomorus* were evaluated for antidiabetic efficacy.

The specific objectives of the study were

1. To determine the principle chemical groups in *Z. chalybeum, X. americana* and *F. sycomorus.*
2. To determine the efficacy of *Z. chalybeum, X. americana* and *F. sycomorus* aqueous extracts in diabetic rats.
3. To determine the effect of *Z. chalybeum, X. americana* and *F. sycomorus* aqueous extracts on biochemical parameters, liver and kidney histology.

CHAPTER THREE

METHODOLOGY

3.1 Study area

Machakos County is located in the Lower Eastern part of Kenya. According to the 2009 National Census, Machakos County had a population of 1,098,584 (Kenya National Bureau of Statistics, 2009). The study area is shown in the map below.

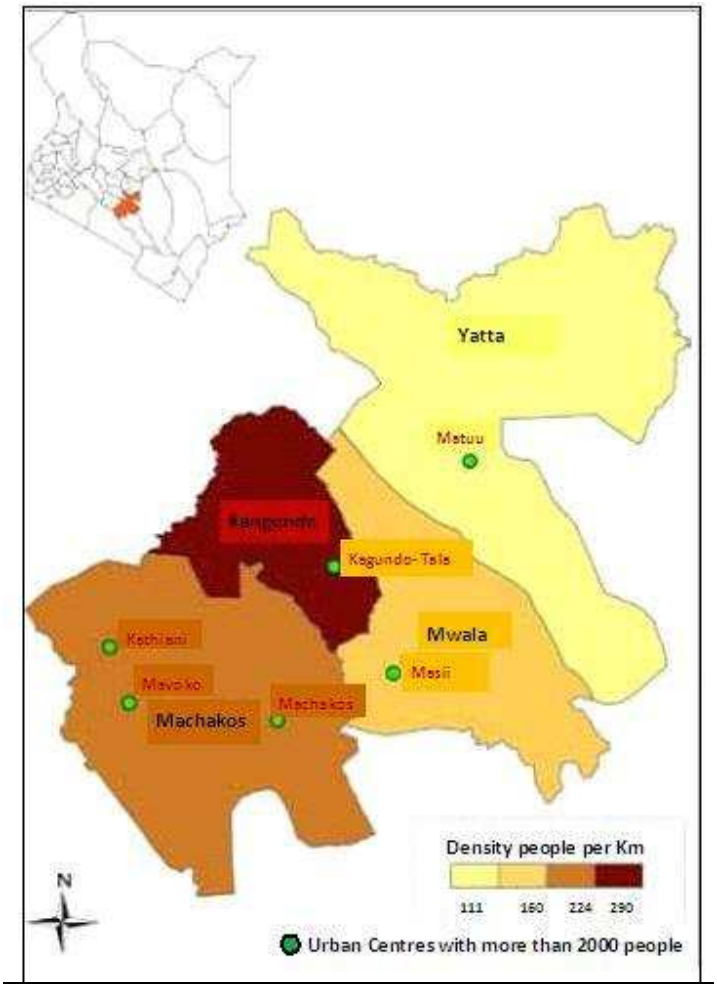


Figure 1: A map of Machakos County

3.2 Collection and identification of plants

Preliminary data on the use of herbs for the management of diabetes in Machakos County was obtained in a meeting with herbalists. The Kamba community is the dominant tribe in this county. The study area was selected based on the extensive utilization of traditional medicine by the community in the area. Information was collected using semi structured questionnaires and also using focus group discussions. The medicinal plants were identified *in situ* by the herbalists during a guided tour of the study area. Plant specimens were collected and placed in a plant press awaiting botanical identification. The specimens were then identified at the University of Nairobi herbarium, in the Department of Botany where voucher specimens were also deposited.

3.3 Preparation of plant extracts

Harvesting was done on a dry day and plants harvested manually and washed thoroughly in running water. Cleaned plant materials were then dried in the shade for one week. The completely dried material was weighed and ground into powder using an electric mill.

For aqueous extraction, 100 g of powder was extracted in 1litre distilled water for 25 minutes using a hot plate. The decoction extract was then filtered and centrifuged at 5000rpm for 10 min and the supernatant collected. This procedure was repeated twice. The supernatant collected was pooled together and concentrated to make the final volume. The extract was freeze dried (Christ Beta 336, Martin Christ Freeze Dryers, Osterode, Germany) and stored at 4º C awaiting phytochemical screening and efficacy and toxicity evaluation.

3.4 Phytochemical screening

The plants were screened for principle chemical groups using the following standard methods.

3.4.1 Test for alkaloids

The presence of alkaloids was determined by first dissolving 0.02 g of extract in 1 ml methanol, filtering the mixture, followed by boiling the extract with 2 ml of 1% hydrochloric acid for 5 minutes. Five drops of Dragendorff's reagent was then be added into the extract. Formation of an orange precipitate indicated the presence of alkaloids (Salehi-Surmaghi *et al*., 1992).

3.4.2 Test for tannins

Half a gram (0.5g) of the water extract (crude dry powder) was dissolved in 2ml of distilled water and filtered. Two drops of ferric chloride was then added to the filtrate. A blue black precipitate indicated the presence of tannins (Segelman *et al*., 1969).

3.4.3 Test for cardiac glycosides

Keller-kiliani test was used to assess the presence of cardiac glycosides. A hundred milligrams (100mg) of crude dry powder of the plant was treated with 1ml of glacial acetic acid containing one drop of 5% ferric chloride ($FeCl_3$) solution. To this solution, 1ml of concentrated sulphuric acid was under-layered. The appearance of a brown ring at the interface of the two layers with the lower acidic layer turning blue green upon standing for a few minutes indicated the presence of cardiac glycosides (Ajaiyeobu, 2002).

3.4.4 Test for steroids

Liebermann-Burchard reaction was used to assess the presence of steroids. A chloroform solution of 0.5g of the crude dry powder of the plant was treated with 0.5ml of acetic anhydride and 2 drops of concentrated sulphuric acid added down the sides of the test tube. A blue green ring indicated the presence of sterols, while colour change from pink to purple indicated triterpenes (Brain and Turner, 1995).

3.4.5 Test for saponins

The presence of saponins was determined by frothing test. Half a gram (0.5g) of the plant extract was shaken in 5ml of distilled water and allowed to stand for 10 minutes. Stable froth more than 1.5cm and persisting for at least 30 minutes was indicative of saponins (Kapoor *et al*., 1969).

3.4.6 Test for flavonoids and flavones

One gram of extract was dissolved in 10 ml distilled water and then filtered using Whatman filter No.1. 10 mg magnesium turnings were then added into 1 ml of the filtrate, followed by the addition of 0.05 ml concentrated sulphuric acid. The presence of magenta red observed within three minutes confirmed the presence of flavonoids, while orange colour indicated presence of flavones (Brain and Turner, 1995).

3.5 Determination of antidiabetic efficacy

3.5.1 Ethical Approval

The efficacy study was conducted at the rodent facility, Institute of Primate Research (IPR), Karen, Kenya. Approval for the study was obtained from the Institutional Review Committee, Institute of Primate Research.

3.5.2 Experimental animals

Eighty, 10 week old, male Wistar rats were purchased from the University of Nairobi. The rats were housed in groups of five in plastic cages with stainless steel covers. They were acclimatized for 3 weeks at room temperature (20–25°c) under a 12/12 h light/dark cycle. All rats received standard rat chow (Unga feeds^{tm}) and distilled water *ad libitum* during acclimatization and also throughout the experimental period. The acclimatized rats were randomized using a table of random numbers and assigned to the experimental groups in sets of five animals per group as shown in Table 3.1

Table 3.1: **Experimental groups: Normal and diabetic rats were given *Z. chalybeum*, *F. sycomorus*, *X. americana* or Glibenclamide at doses of 10, 100 or 1000mg/kg body weight orally**

Group Number	Streptozotocin 45mg/kg bwt	*Z. chalybeum*	*X. americana*	*F. sycomorus*	Glibenclamide 10mg/kg bwt
1	X	X	X	X	X
2	√	X	X	X	X
3	√	10mg	X	X	X
4	√	100mg	X	X	X
5	√	1000mg	X	X	X
6	√	X	10mg	X	X
7	√	X	100mg	X	X
8	√	X	1000mg	X	X
9	√	X	X	10mg	X
10	√	X	X	100mg	X
11	√	X	X	1000mg	X
12	√	X	X	X	√
13	X	√	X	X	X
14	X	X	√	X	X
15	X	X	X	√	X

Diabetic rats were either given Glibenclamide or one of the various doses of the plant extracts. Particular treatments given are indicated by (√), the cross (X) indicates that animals in that group were not given that particular treatment.

The respective extracts, doses 1000mg/kg bwt, 100mg/kg bwt and 10mg/kg bwt were reconstituted in distilled water and administered daily at 0900 hrs via stomach tube. This was done for fourteen days. Rats in groups 1 and 2 received distilled water, while rats in group 12 received Glibenclamide (10mg/kg bwt) at a concentration of 200µg/ml for fourteen days. Rats were weighed on a weekly basis using a weighing balance (Sartorius, GMBH GOTTINGEN, Type L2200P, Germany). Weights were expressed in grams.

3.5.3 Induction of diabetes

Rats in groups 2-12 were fasted overnight and injected intraperitoneally with streptozotocin (STZ, Sigma Aldrich, USA) at a dose of 45mg/kg body weight to induce diabetes. The streptozotocin powder was reconstituted in sterile 0.9% Sodium chloride, at a concentration of 7.5mg/ml. A drop of blood was collected from the tail vein on day 3, 7 and 12 after injection and glucose levels determined using a glucometer (Softstyle®, Chemlabs, Kenya) to confirm stable hyperglycemia. Rats with glucose levels greater than 14mmol/L were considered diabetic and used for the efficacy study.

3.5.4 Determination of plasma glucose

Blood was obtained by a prick on the lateral tail vein and blood glucose determined using a glucometer (SoftStyle® Chemlabs, Kenya). Results were expressed in mmol/L. The rats were sampled for a further seven days after extract administration to assess long term glucose control.

Blood for determination of plasma glucose was obtained from the tail vein and the clinical profile of the animals described.

3.5.5 Oral glucose tolerance test (OGTT)

The OGTT was performed to determine the short-term effect of the extracts on glucose control at the end of extract administration (day 14). Rats in all groups were fasted overnight and then administered 2 g glucose kg−1 body weight orally. A drop of blood was withdrawn from the tail vein before (0 min) and 30, 60, 90 and 120 min after administration of glucose solution. Blood glucose was measured using a glucometer and results expressed in mmol L−1.

3.5.6 Biochemical parameters

Blood for biochemical evaluation was collected via cardiac puncture at euthanasia. Samples approximately 5mls were collected into serum tubes. The blood was allowed to clot and left for 10 minutes at room temperature for serum to form. Serum was separated by centrifugation at 3000rpm for 10 minutes and stored at−20ºC until required for analysis. Liver enzymes; aspartate aminotransferase, alanine aminotranferase and alkaline phosphatase, creatinine and blood urea nitrogen were determined using commercial kits (Humalyzer 2000, Human Diagnostics®, Germany) according to the manufacturers' protocol.

3.5.7 Pathological examination

The animals were euthanized in a carbon dioxide chamber and the pancreas, liver and kidneys removed and fixed in a 10% solution of formaldehyde. The fixed tissues were then dehydrated in graded concentrations of alcohol (50-100%), cleared in xylene and embedded in paraffin wax. The sections (5µm) from each of the tissues were examined using a microscope (10x and 40x) after staining with hematoxylin and eosin dye.

3.6 Statistical analysis

Data was expressed as mean ±standard error of mean (SEM). Two-way analysis of variance (ANOVA, GraphPad Prism 5) was used to determine differences in means between groups. Values were considered significantly different at the level of $P < 0.05$.

CHAPTER FOUR

RESULTS

4.1 Meeting with herbalists

A total of seven traditional health practitioners were recruited as resource persons for the survey. The interviewed (5 male, 2 female) had a mean age of 51.6 ± 3.27 years. Three of the interviewees had attained primary education while four had attained secondary education. Six of the THPs interviewed acquired the traditional medical knowledge from members of the family and one through divine visions. The duration of practice ranged from 5-40 years. All the interviewed were affiliated to the Ukambani Herbalists Association.

4.1.1 Traditional Health Practitioners' knowledge of diabetes

The interviewees had good knowledge of diabetes on the basis of acceptable clinical symptoms such as frequent thirst, frequent urination, fatigue, dizziness and problems with vision. The interviewees also relied on laboratory reports and also reports from patients who had confirmed cases of diabetes and were already on conventional treatment. Five of the interviewees associated diabetes with family history.

4.1.2 Plant species used to treat diabetes mellitus

Nineteen plants were mentioned as being used for treatment of diabetes mellitus in Machakos and its surrounding towns. Out of these, a total of sixteen plant species, distributed across 13 families were identified *in situ* with the assistance of the herbalists and specimens collected. The

plant species, family, vernacular names, the parts used, and mode of preparation are shown in Table 4.1. The most frequently mentioned plants were *Zanthoxylum chalybeum*, *Ximenia americana* and *Ficus sycomorus*. These were selected for phytochemical analysis and efficacy and safety evaluation.

The family Asteraceae was represented by the highest number of species (three species) and Fabiaceae by two species. The rest were represented by one species each (11 families).

Table 4.1 Plants used for management of diabetes mellitus in Machakos County

Family	Plant Species	Local name	Frequency of Mention (n=7)	Part of plant used	Preparation and Use
Rutaceae	*Zanthoxylum chalybeum* Engl.	Mukenea	4	Stem bark, roots, leaves, seeds	Half a teaspoonful in hot water 2 times daily
Olacaceae	*Ximenia americana* Linn	Mutula	4	Leaves, seeds, roots, stem bark	3 teaspoonfuls in 4 glasses hot water, take one glass 3 times a day for 14 days
Lamiaceae	*Ocimum kilimandscharium* Guerke	Mukandu	3	Leaves, whole plant	Boil 1litre water, add 4 teaspoons
Simaroubaceae	*Harrisonia abyssinica* Oliv	Mukilyulu	1	Leaves, roots, seeds	Half a teaspoonful in hot water 2 times daily
Polygonaceae	*Oxygonum stuhlmannii* Dammer	Song'e	1	Whole plant	One teaspoonful in hot water 2 times daily
Amaranthaceae	*Amaranthus caudatus* Linn	Musavula	1	Whole plant	One teaspoon in hot water for 4 weeks (mixed with several others)
Fabaceae	*Erythrina abyssinica*	Muvuti	2	Stem bark,	Dose; 3 teaspoonfuls in 4 glasses of hot water.

	Lam			leaves	Take one glass 3 times a day for 14 days
Asphodelaceae	*Aloe secundiflora* Engl.	Kiluma	3	Stem, leaves	Dose: 1 teaspoonful in 4 glasses of hot water. One glass in the morning and evening for 14 days
Asteraceae	*Launea cornuta* Hochst	Muthunga (small)	3	Whole plant	Dose: 2 teaspoonfuls in 4 glasses of hot water. Take 1 glass twice daily for 14 days.
Asteraceae	*Sonchus asper* (L.) Hill	Muthunga (Giant)	3	"	"
Moraceae	*Ficus sycomorus* Linn.	Mukuyu	4	Stem bark, leaves	Dose: 2 teaspoofuls in 4 glasses of hot water, take one glass twice daily
Fabaceae	*Acacia mellifera* Vahl.	Muthiia	3	Stem bark	Boil 2 teaspoons in 500mls water
Asteraceae	*Bidens pilosa* Linn.	Munzee	2	Flowers, whole plant	1 tablespoonful in hot water for very high hyperglycemia, 3 times daily. For mild hyperglycemia, 3 teaspoons in hot water 3 times daily
Tiliaceae	*Grewia bicolor* Juss.	Mulawa	1	Stem bark	2 teaspoonfuls to 1 litre. Boil for 10 minutes
Verbenaceae	*Lantana virbunoides* Forssk.	Mukeny'a	1	Whole plant	2 tablespoonfuls to 1 litre hot water
Labiatae	*Ocimum suave* Linn.	Mwenye	2	Leaves, whole plant	2 tablespoonfuls to 1 litre hot water

*All plants/ plant parts were dried under shade and ground into powder before being constituted

4.1.3 Plant parts used

Majority of the plants were used as the entire plant (28.6%), followed by the stem bark (25%), leaves (21.4%) and root bark and seeds (10.7% each) while the flowers accounted for 3.6%. This information is presented in Table 4.2 below.

Table 4.2: Frequency of plant part used for the preparation of traditional diabetes remedies

Plant parts used	Number of plant species	Percentage (%)
Whole plant	8	28.6
Stem bark	7	25
Leaves	6	21.4
Root bark	3	10.7
Seeds	3	10.7
Flowers	1	3.6

4.1.4 Herbal medicinal preparations and administration

The plants are used either as mixtures or as single plants. To prepare, the plant parts are first harvested then dried in the shade. The completely dried plant(s) are then ground into powder. A specific quantity of the powder is then mixed in hot water or boiled in water for about 10 minutes. The resulting decoction or infusion is taken several times a day, depending on the prescription from the particular traditional health practitioner. These medicines are prepared when required and most interviewees did not preserve the medicines. The herbal medicines are administered orally and the most commonly mentioned quantities and frequency of administration were one teaspoon three times a day, one tablespoonful three times daily and one cup three times daily. The duration of treatment ranged from one week to four months. Most of the interviewees reported that the plants used were not associated with any toxicity. There was only one mention of stomach ulcers related to use of some of the plants at high doses. This was remedied by a concoction prepared by the traditional health practitioner to reduce the level of acidity. However, interviewees advised their patients to avoid alcohol, meat, sugary foods and salt.

4.2 Phytochemical Analysis

The aqueous stem bark extracts of *Z. chalybeum*, *F. sycomorus* and *X. americana* contain several secondary metabolites as shown in Table 4.3 below.

Table 4.3: Secondary metabolites from *Z. chalybeum*, *F. sycomorus* and *X. americana* crude stem bark extracts

Compound	*Z. chalybeum*	*F. sycomorus*	*X. americana*
Alkaloids	+	-	-
Flavonoids	+	+	+
Steroids	-	-	-
Terpenoids	+	+	+
Saponins	+	-	-
Tannins	+	+	+
Phenols	+	-	+
Glycosides	+	+	+

+ Present

- Absent

4.3 General characteristics of the animals

Three days after administration of streptozotocin (45mg/kg bwt IP) the rats appeared lethargic and displayed restricted movement, however their demeanor improved in the weeks during and after treatment. Rats classified as diabetic had hyperglycemia ≥ 14mmol/L. Diabetic rats also displayed polyuria, polydipsia and weight loss three days after induction. Control rats were active throughout the study period.

4.4 Effect of *Z. chalybeum, F. sycomorus* and *X. americana* stem bark extracts on fasting blood glucose levels

At baseline, before induction of diabetes, blood glucose levels were not significantly different in the various experimental groups, with levels of 3.4 - 4.6mmol/L. Three days after administration of streptozotocin (45mg/kg bwt) the rats exhibited hyperglycemia (range 14-38.4mmol/L). The results are presented in Figures 2, 3 and 4 below.

Administration of the extract of *Z. chalybeum* stem exhibited significant decreases in fasting blood glucose in diabetic rats at the three dose levels of 10mg, 100mg and 1000mg per kg body weight ($P<0.05$). This difference was not significant for all three doses of the extract, compared to the rats given Glibenclamide (10mg/kg bwt). Similarly, there was no significant difference between the extract fed diabetic rats and the normal controls or the non-diabetic rats that were given 1000mg/kg bwt of the extract ($P<0.05$). However, blood glucose levels of the rats given 10mg/kg bwt of the extract were significantly higher ($P<0.05$) compared to both the normal

controls and the normal rats given *Z. chalybeum* at 1000mg/kg body weight. There was no significant difference ($P<0.05$) in blood glucose levels at different doses of the extract for the diabetic rats. The blood glucose levels of normal rats were not changed. Untreated diabetic rat blood glucose levels were significantly ($P<0.05$) and continuously elevated throughout the experimental period. Figure 2 below summarizes these results.

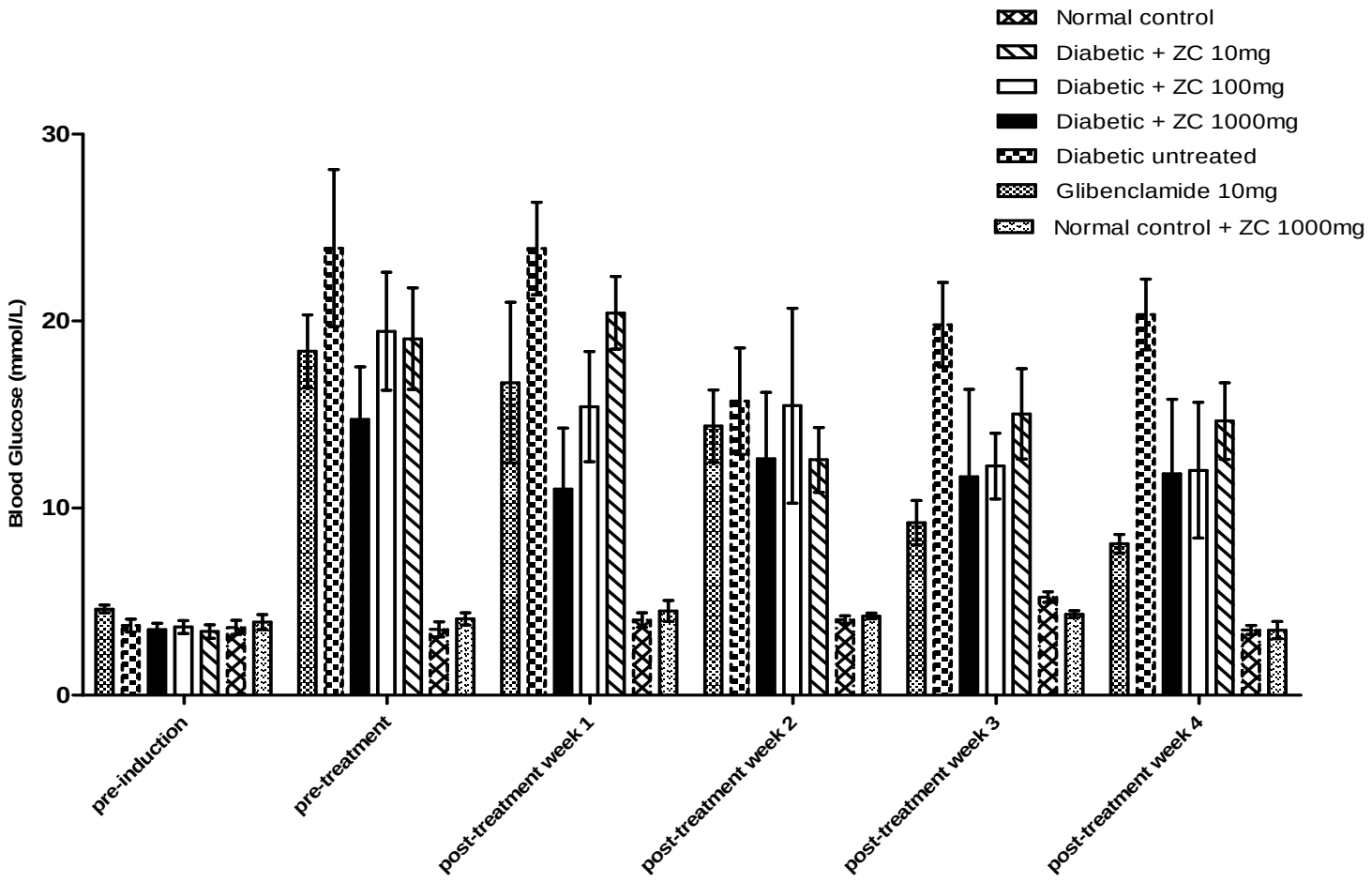


Figure 2: Effect of *Z. chalybeum* on fasting blood glucose levels in normal and diabetic rats

F. sycomorus stem bark extract significantly reduced glucose levels in diabetic rats ($P<0.05$) at doses of 100mg and 10mg/kg body weight compared to untreated diabetic rats. However, the decrease at the dose of 1000mg/kg body weight was not significantly different from the untreated diabetic group. Comparing these levels with the Glibenclamide treated group there was no significant difference ($P<0.05$) with the extract treated groups (100mg and 10mg/kg bwt) though levels were significantly lower compared to the diabetic rats given 1000mg/kg body weight of extract. Similarly, blood glucose levels of the diabetic rats given 1000mg were significantly higher compared to the diabetic rats given lower doses of the extract (100mg and 10mg/kg bwt) ($P<0.05$). Levels of blood glucose in the diabetic 1000mg/kg bwt group were also significantly higher compared to the normal control groups. In contrast, lower doses of the extract reduced blood glucose levels such that these were not significantly different compared to the normal controls ($P<0.05$). A summary is presented in Figure 3.

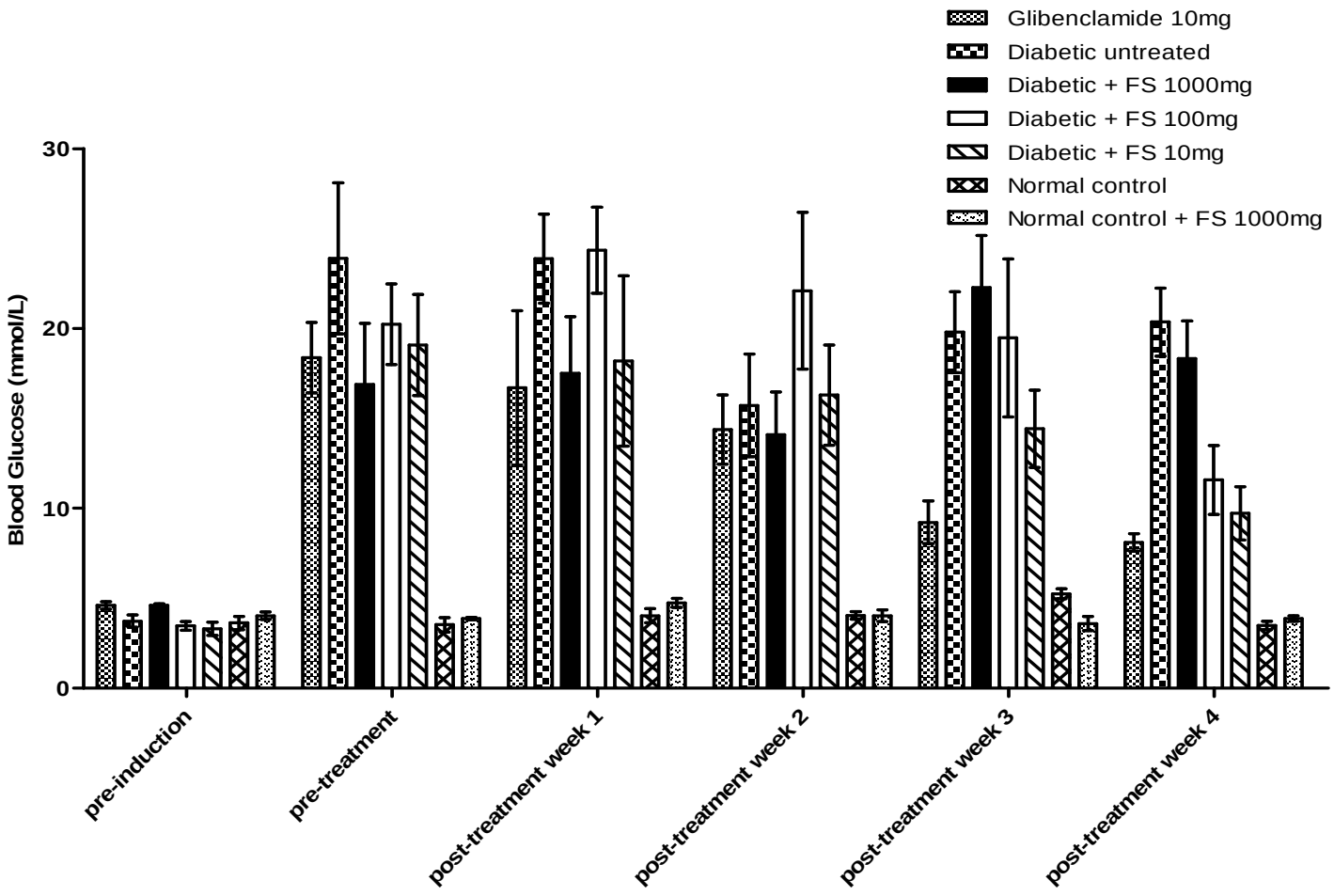


Figure 3: Effect of *F. sycomorus* stem bark extract on fasting blood glucose levels in normal and diabetic rats

X. americana stem bark extract at the three dose levels employed, reduced blood glucose to levels that were not significantly different ($P<0.05$) compared to the Glibenclamide group. Additionally at 100mg and 10mg/kg bwt, blood glucose levels were significantly reduced compared to the untreated diabetic group. The difference between the three dose levels was not significantly different, as was the difference compared to normal control groups ($p<0.05$). These results are summarized in Figure 4 below.

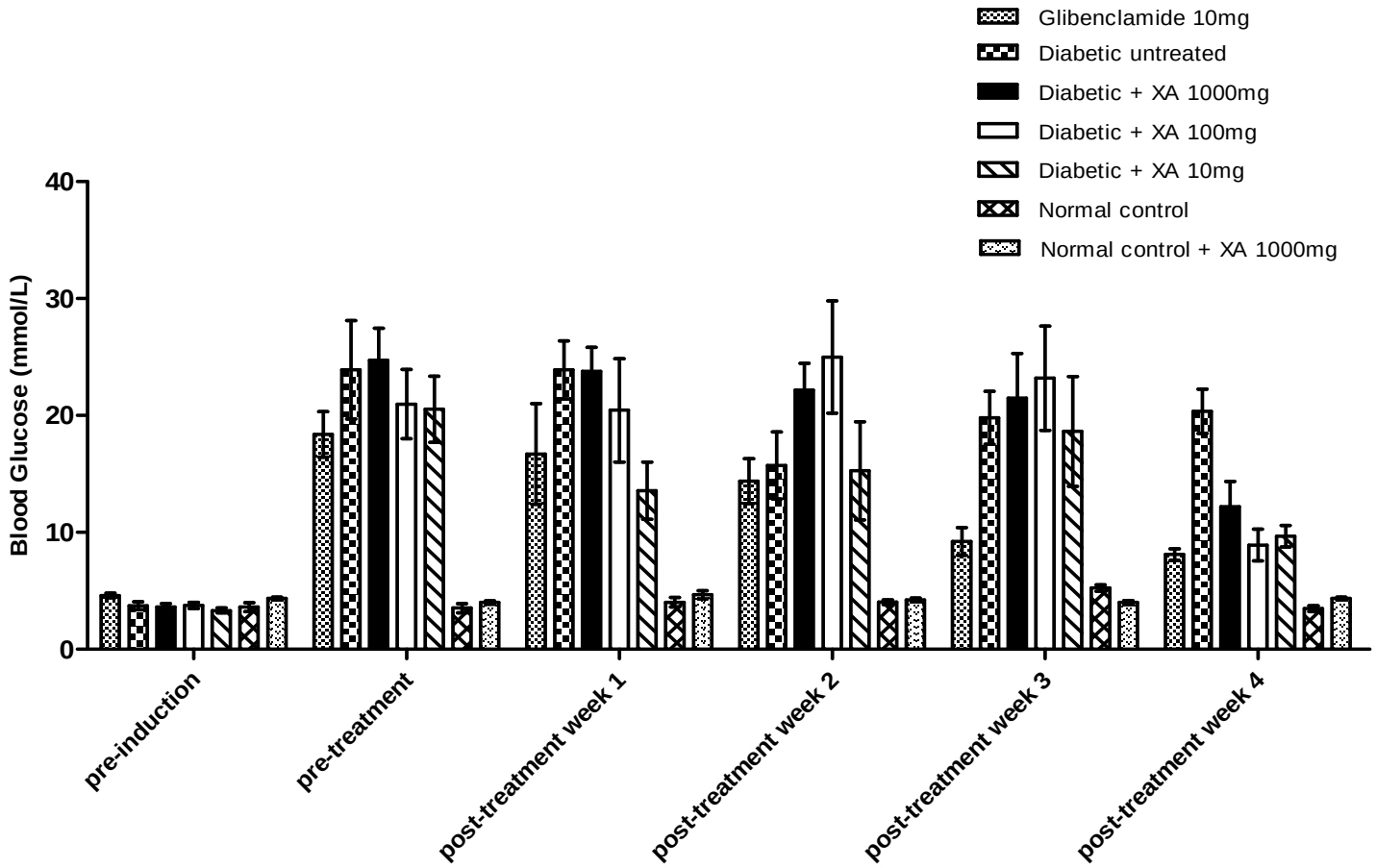


Figure 4: Effect of *X. americana* stem bark extract on fasting blood glucose levels in normal and diabetic rats

4.5 Effect of *Z. chalybeum*, *F. sycomorus* and *X. americana* stem bark extracts on oral glucose tolerance

Zanthoxylum chalybeum administration 30 minutes prior to glucose loading resulted in a gradual reduction in glucose levels, but this was not statistically significant compared to the diabetic controls ($P< 0.05$). Results of the OGTT are shown in Figure 5 Glibenclamide (10mg) did not reduce the levels significantly compared to the untreated diabetic controls. The results were comparable to those of the three dose levels of the extract. The gradual decrease in blood glucose levels was not dose dependent. The diabetic control had the highest reduction (39.8%) in blood glucose 120min after glucose load, followed by the diabetic (10mg) group (38.9%). There was no significant difference in the normal controls ($P< 0.05$).

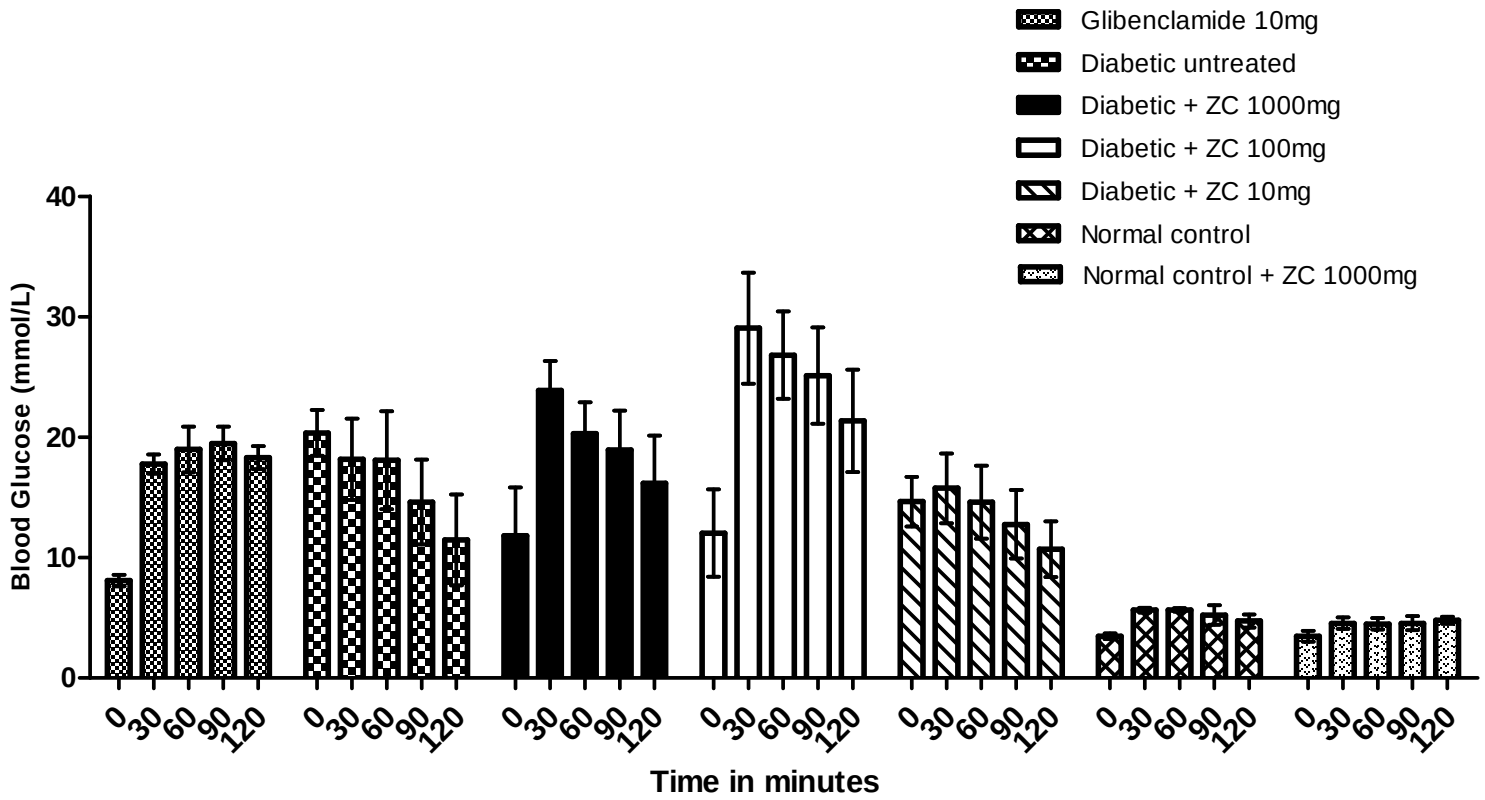


Figure 5: Effect of *Z. chalybeum* extract on fasting blood glucose level after glucose load in normal and diabetic rats

For *F. sycomorus* there was no significant difference between the Glibenclamide treated rats and the extract treated diabetic rats except at 2 hours post glucose loading with the diabetic rats given 1000mg/kg body weight of the extract ($P<0.05$). Similarly, the difference between the three dose levels was not statistically significant. This information is presented in Figure 6.

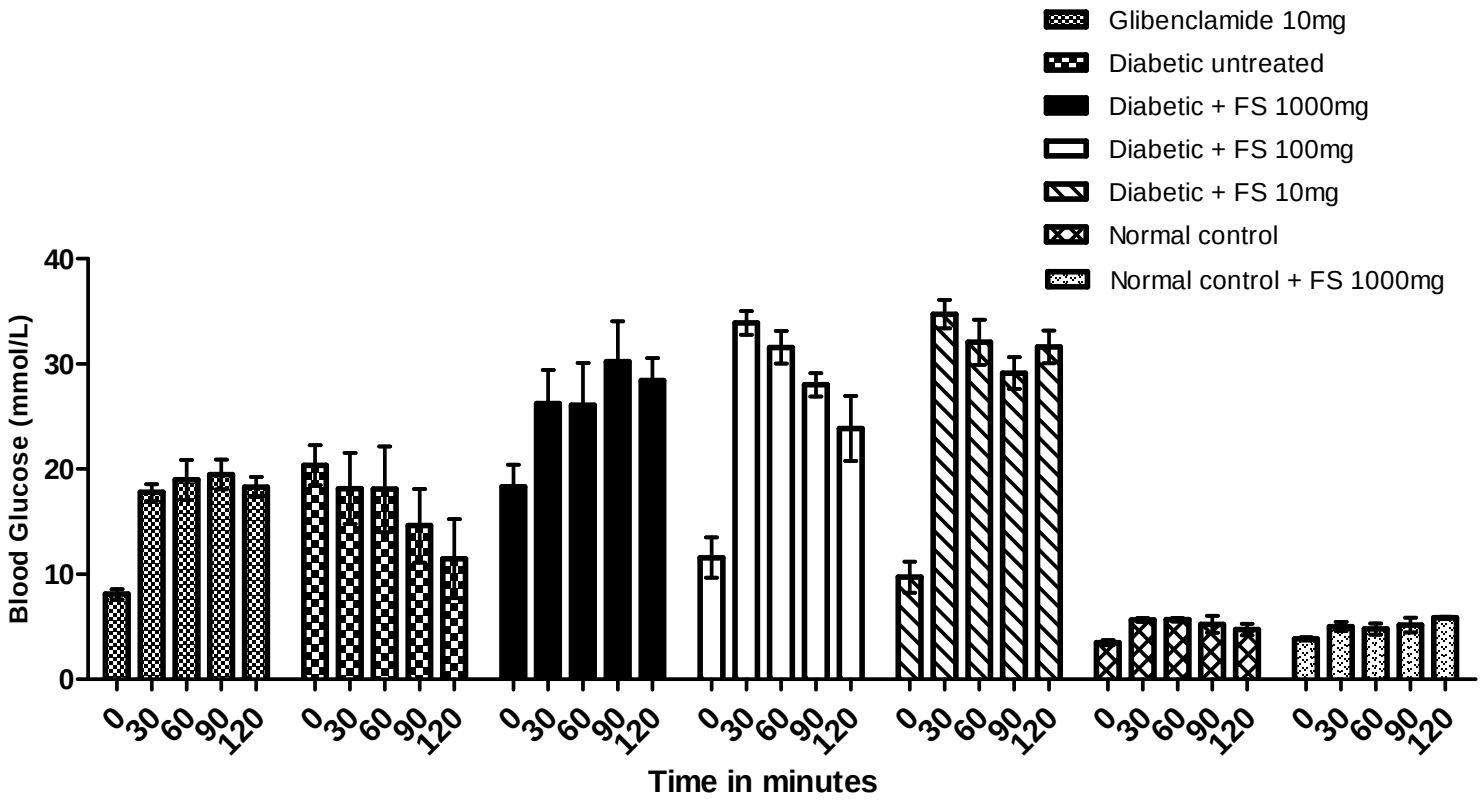


Figure 6: Effect of *F. sycomorus* extract on fasting blood glucose level after glucose load in normal and diabetic rats

X. americana resulted in a gradual decrease in blood glucose levels after glucose load. This decrease was however not statistically significant compared to the control groups ($P<0.05$). This data is shown in Figure 7.

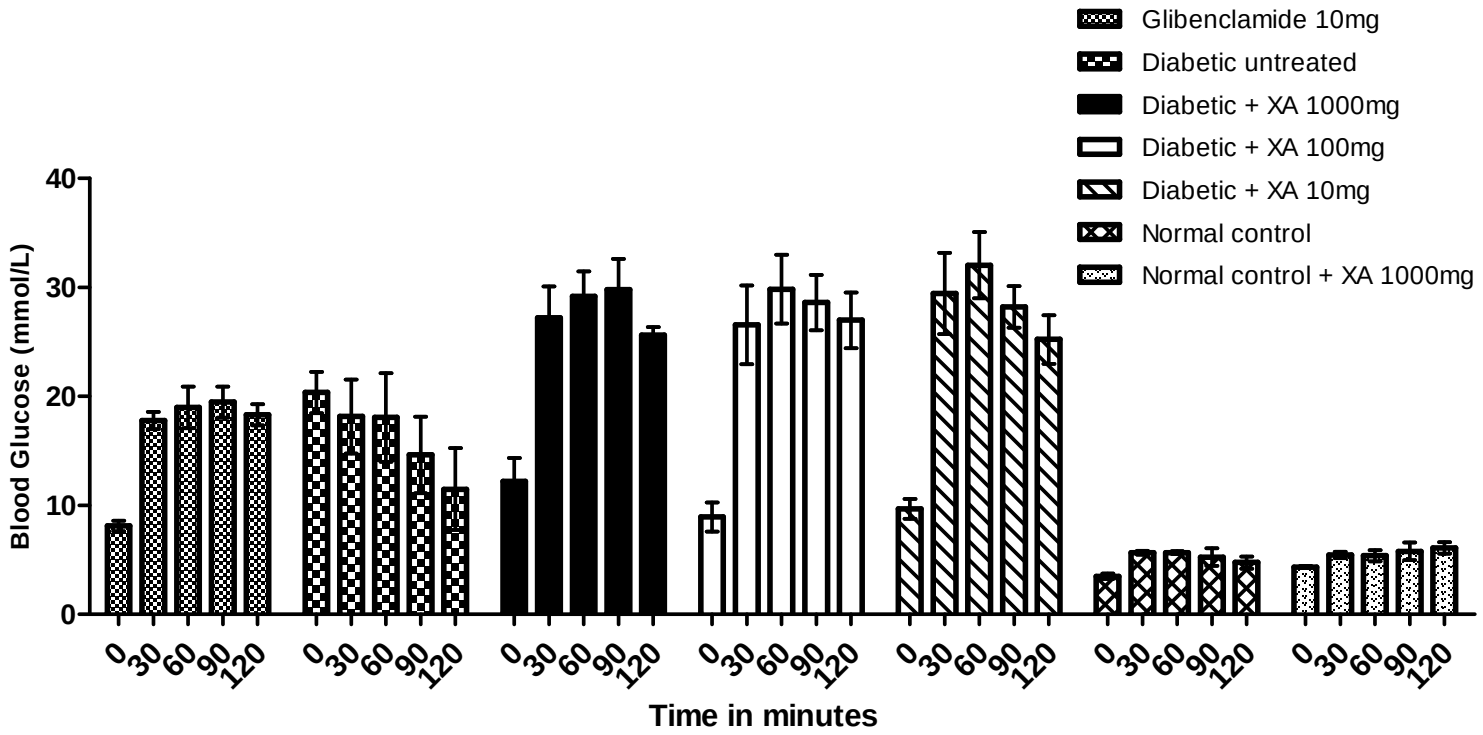


Figure 7: Effect of *X. americana* extract on fasting blood glucose level after glucose load in normal and diabetic rats

4.6 Effect of *Z. chalybeum*, *F. sycomorus* and *X. americana* stem bark extracts on body weight

There was a significant loss in the body weight of treated and untreated diabetic rats 1 week after induction of diabetes (Figures 8, 9 and 10). This decrease was greatest in the untreated diabetic group (36.44%). *Z. chalybeum* extract treated diabetic animals experienced a comparatively decreased weight loss which was dose dependent at 15.45%, 24.38% and 26.63% for 1000mg/kg, 100mg/kg and 10mg/kg treated animals respectively, compared to the untreated diabetic rats at 36.44% . The weight loss in the Glibenclamide treated rats (16.33%) was not significant compared to the normal control groups 4 weeks post treatment, although this difference was significant when compared with the normal control rats that were given 1000mg/kg bwt of the extract. The weights of the extract treated diabetic rats, at all three doses were not significantly different compared to the Glibenclamide treated rats ($P<0.05$). Extract treated normal controls had a higher weight gain (20.79%) compared to the untreated normal controls (2.82%). Figure 8 below summarizes this information.

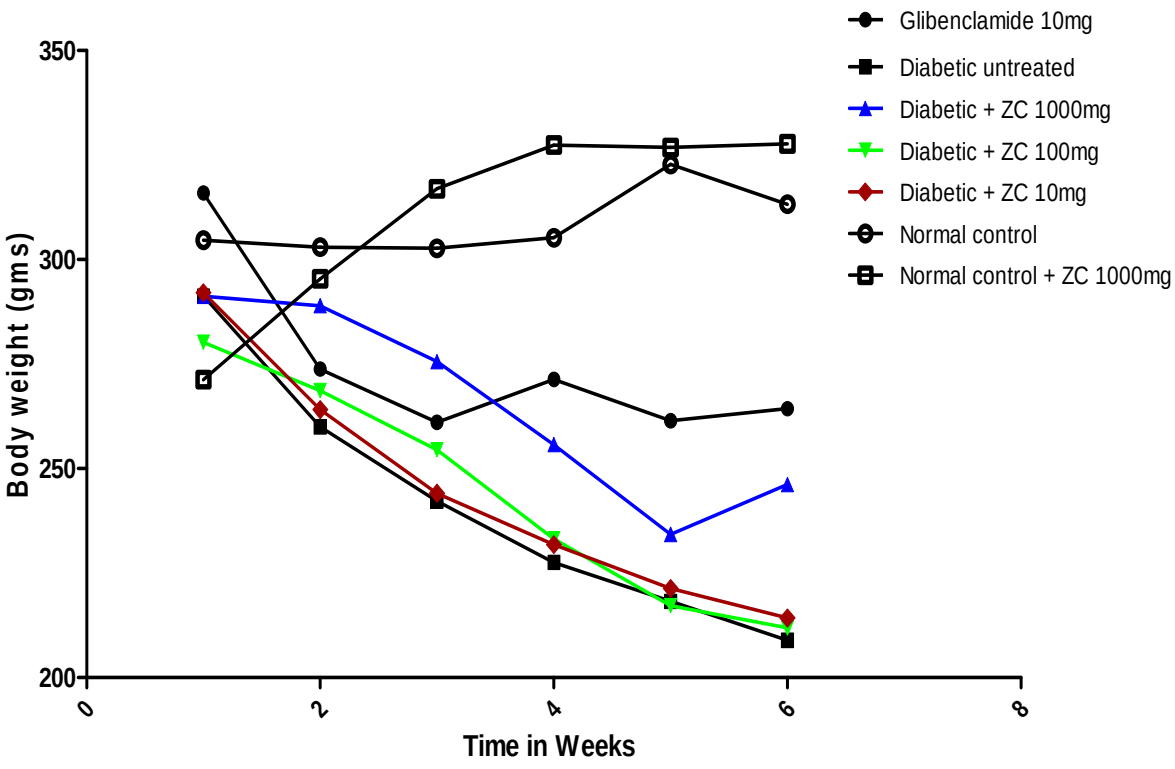


Figure 8: Effect of *Z. chalybeum* extract on body weight in normal and diabetic rats

The *F. sycomorus* treated diabetic rats had a weight loss of 17.52%, 33.89% and 30.17% respectively for 1000mg, 100mg and 10mg/kg bwt, which was higher compared to the Glibenclamide treated rats (16.33%). This decrease was significant for 100mg and 10mg/kg bwt treated rats compared to the Glibenclamide group ($P<0.05$). The extract treated normal control group had a higher weight increase (15.6%) compared to the untreated normal controls (2.82%), though this increase was not statistically significant ($P<0.05$). A summary is presented in Figure 9 below.

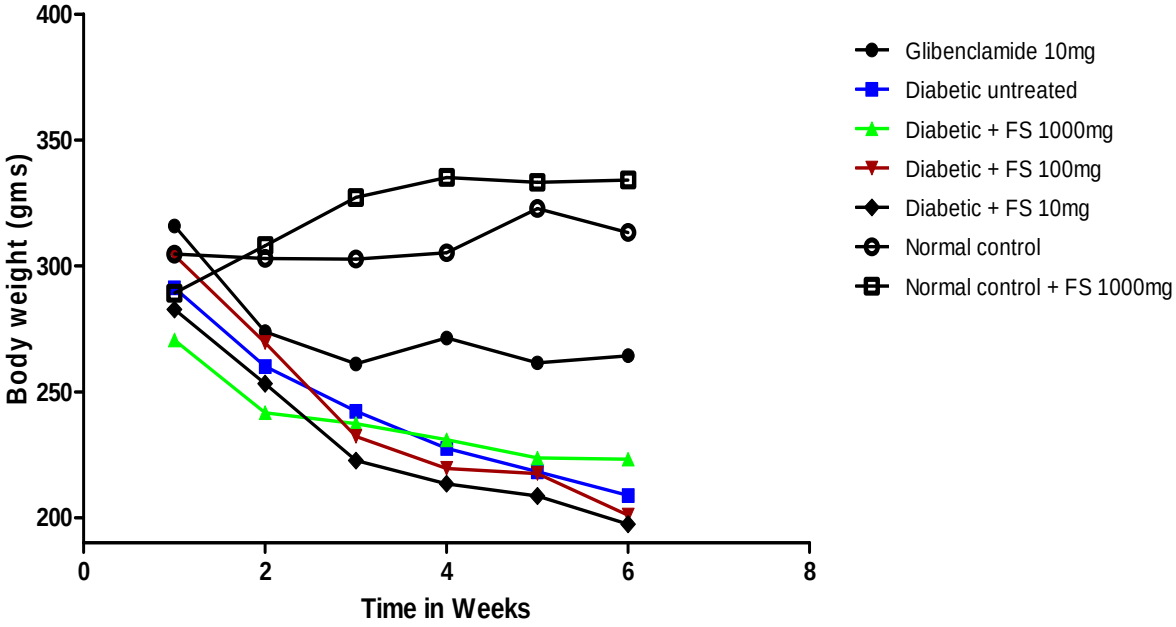


Figure 9: Effect of *F. sycomorus* extract on body weight in normal and diabetic rats

The *X. americana* treated diabetic rats showed a dose dependent decrease in weight loss at 33.03%, 31.88% and 27.17% for 1000mg, 100mg and 10mg/kg bwt respectively. This weight loss was statistically significant ($P< 0.05$) for 1000mg and 100mg/kg bwt when compared with the Glibenclamide group (16.33%). Extract fed normal controls had a higher weight gain (16.63%) compared to the normal controls (2.82%), although this was not significant ($P<0.05$). Figure 10 is a summary of this information.

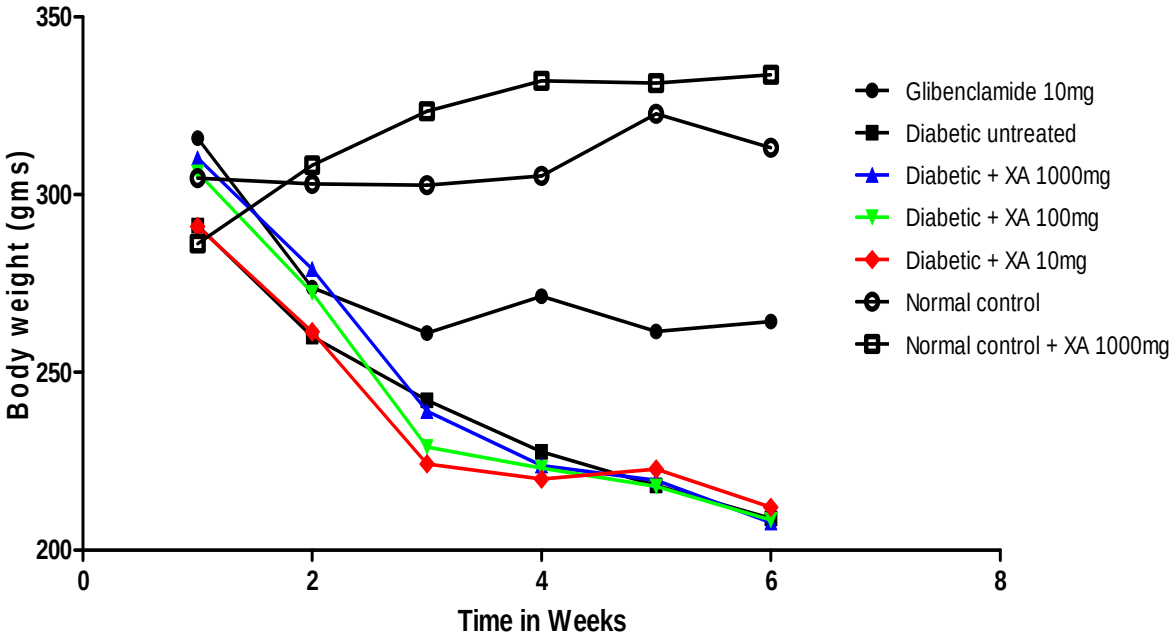


Figure 10: Effect of *X. americana* extract on body weight in normal and diabetic rats

4.7 Effect of *Z. chalybeum*, *F. sycomorus* and *X. americana* stem bark extracts on food intake

Consumption of standard chow was measured daily per cage. Data reported here are mean daily consumption in grams per kilogram of body weight per day. Diabetic rats had significantly higher food consumption compared to non-diabetic rats ($P<0.05$). Administration of extract or Glibenclamide did not reduce these levels back to baseline or normal control levels. There was also no significant difference between the three extract groups ($P<0.05$). Extract treated normal controls had a higher food intake compared to normal controls but this difference was not statistically significant ($P<0.05$). Figure 11 below is a summary of these results.

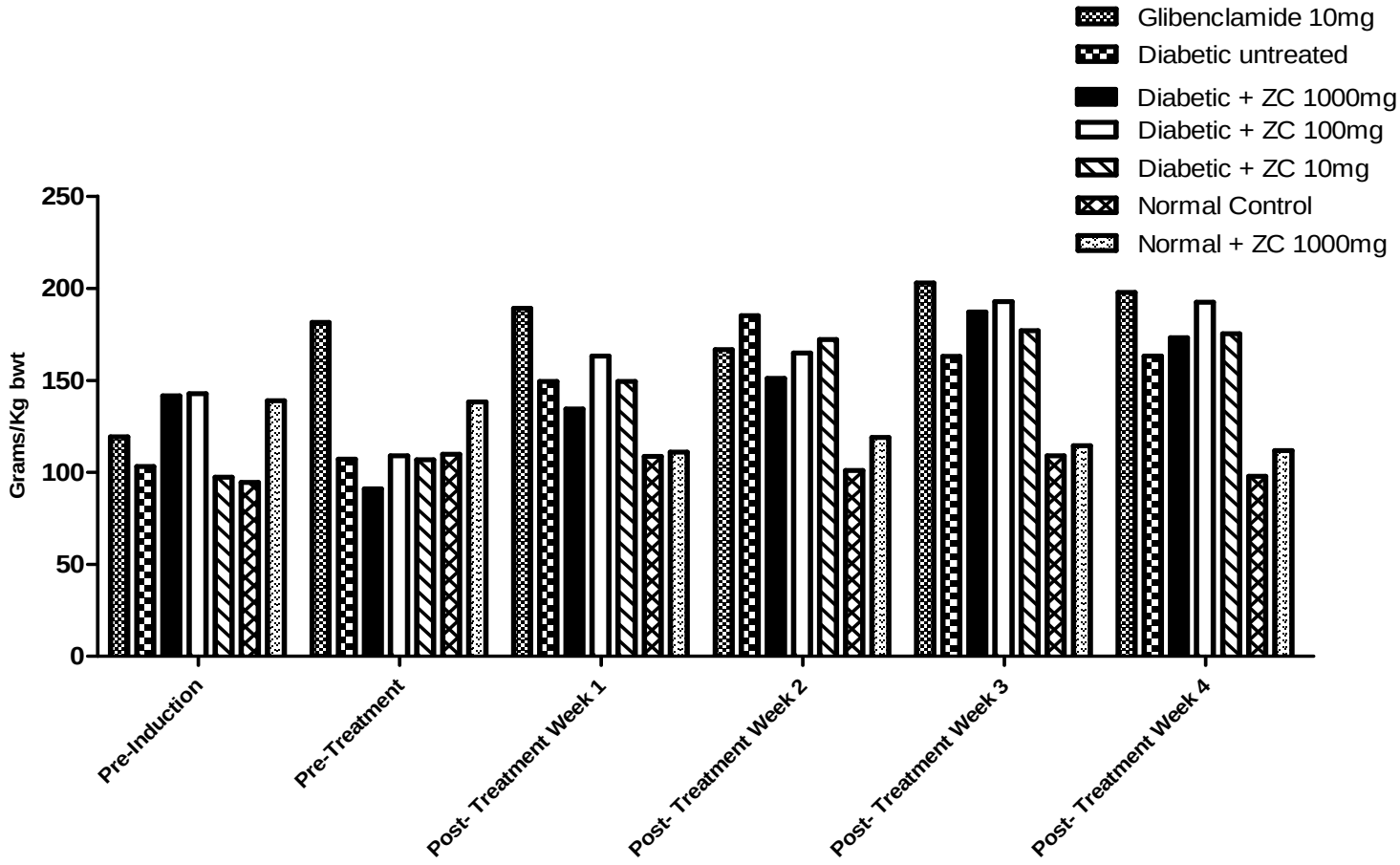


Figure 11: Effect of *Z. chalybeum* extract on food intake in normal and diabetic rats

Diabetic rats treated with *F. sycomorus* stem bark extract had elevated food intake which was significantly higher compared to the normal controls and extract treated normal rats ($P<0.05$). These levels were however not significantly different compared to the untreated diabetic rats. There was no significant difference between the three dose levels ($P<0.05$). Extract treated normal rats showed no significant difference compared to untreated normal controls ($P<0.05$). This information is presented in Figure 12 below.

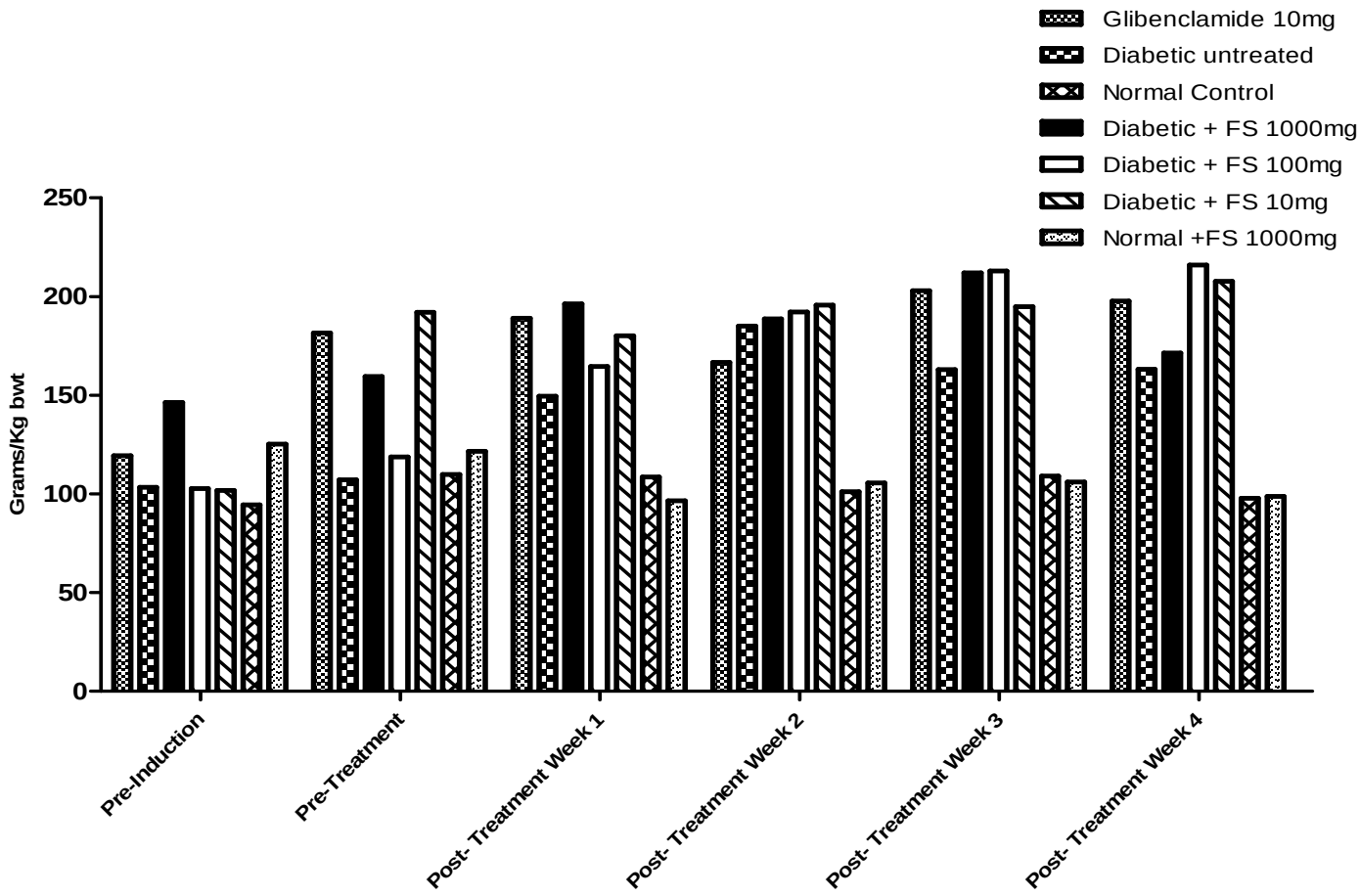


Figure 12: Effect of *F. sycomorus* extract on food intake in normal and diabetic rats

Rats treated with *X. americana* stem bark extract did not have significantly elevated food intake. However, this was significantly higher compared to the normal controls only in the 100mg/kg bwt dose group ($P<0.05$). There was no significant difference between extract treated diabetic rats at the three dose levels ($P<0.05$). Extract treated diabetic rats showed no difference compared to the Glibenclamide treated diabetic rats. Normal controls and extract treated normal controls showed no significant difference ($P<0.05$). These results are presented in Figure 13 below.

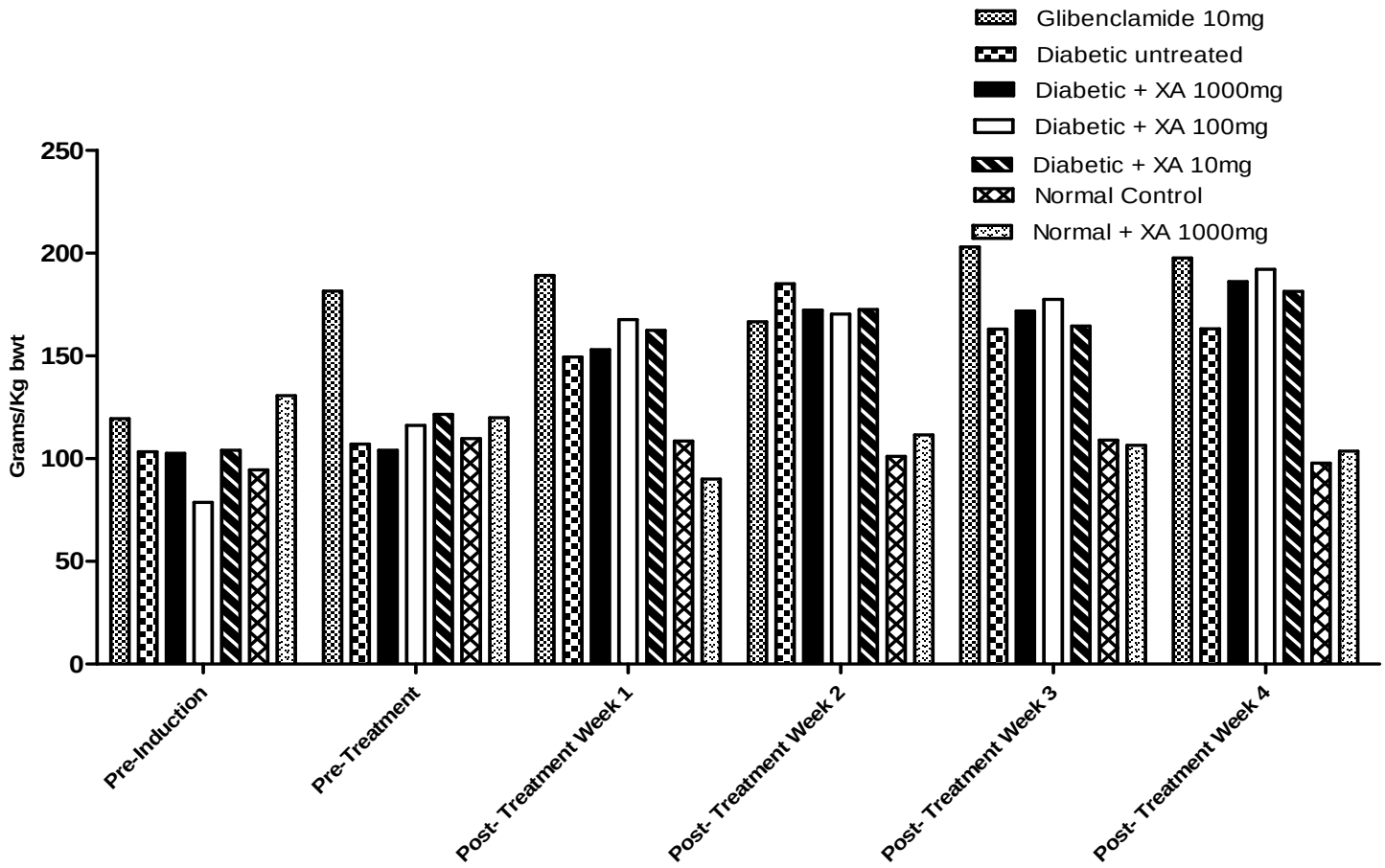


Figure 13: Effect of *X. americana* extract on food intake in normal and diabetic rats

4.8 Effect *Z. chalybeum*, *F. sycomorus* and *X. americana* stem bark extracts on water intake

Water consumption per cage was measured weekly for the duration of the study. Significant differences ($P < 0.05$) in the consumption between diabetic and non-diabetic rats were seen 72 hours post induction and throughout the 28-day post induction period. Extract administration did not reduce these levels back to baseline or normal control levels. Extract administration did not cause a significant difference in water intake in the normal controls ($P<0.05$). Diabetic rats treated with Glibenclamide had significantly elevated water intake throughout the study period ($P<0.05$). A summary is presented in Figures 14, 15 and 16 below. Data are presented as millimeters of water consumed per rat per day.

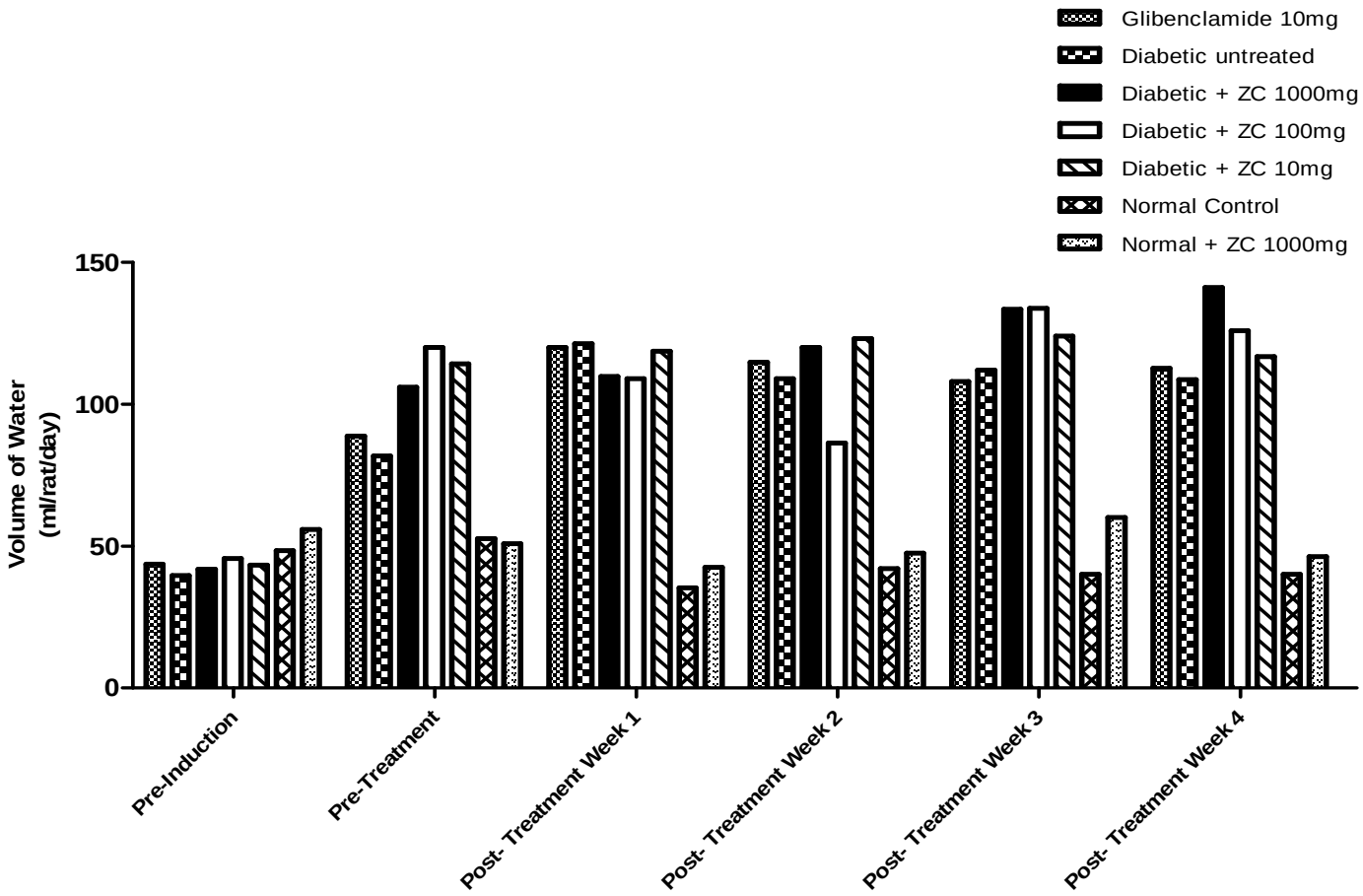


Figure 14: Effect of *Z. chalybeum* extract on water intake in normal and diabetic rats

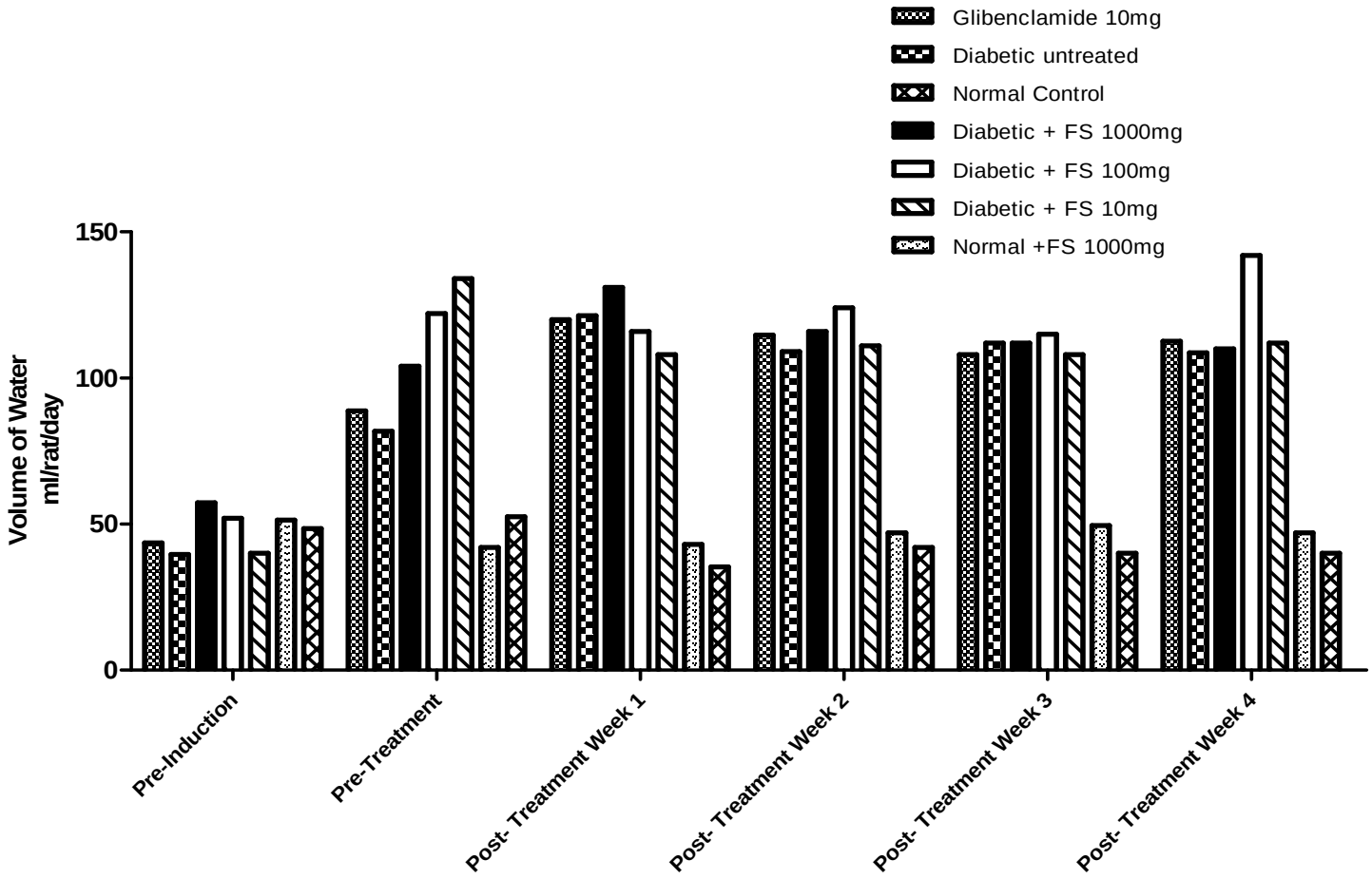


Figure 15: Effect of *F. sycomorus* extract on water intake in normal and diabetic rats

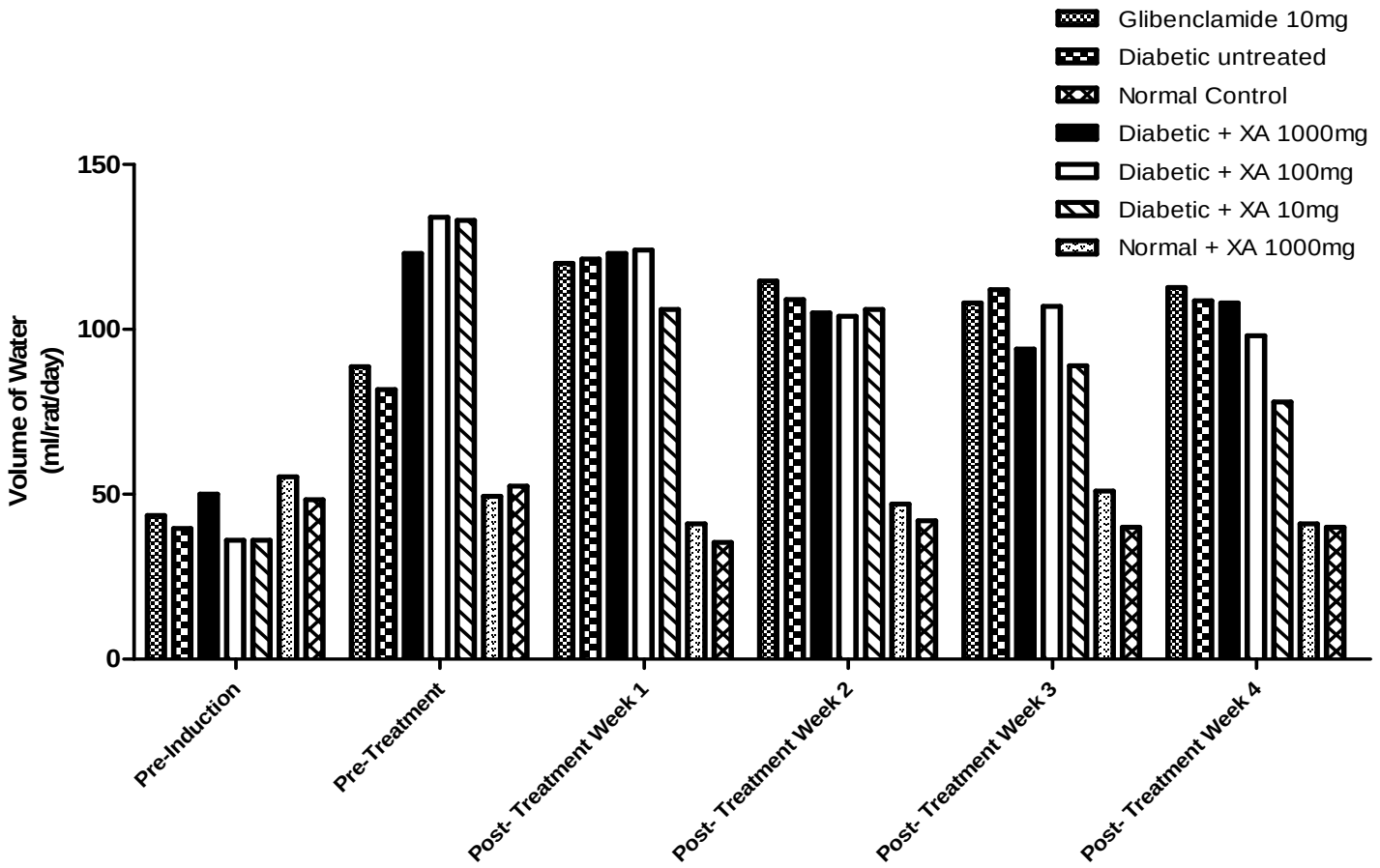


Figure 16: Effect of *X. americana* extract on water intake in normal and diabetic rats

4.9 Effect of *Z. chalybeum*, *F. sycomorus* and *X. americana* stem bark extracts on biochemical parameters glutamic oxaloacetic transaminase, glutamic pyruvic transaminase and alkaline phosphatase

All diabetic rats had significantly elevated alkaline phosphatase levels compared to the normal controls ($P<0.05$). Diabetic rats treated with *Z. chalybeum* and *F. sycomorus* had significantly lower alkaline phosphatase levels compared to untreated diabetic rats ($P<0.05$). For *Z. chalybeum* and *F. sycomorus*, the levels of alkaline phosphatase (ALP) were inversely proportional to dose of extract used with the difference between the doses being statistically significant ($P<0.05$). In contrast extract treated normal rats had significantly lower levels compared to normal controls ($P<0.05$).

Diabetic rats had elevated levels of serum glutamic oxaloacetic transaminase (GOT) and glutamate pyruvate transaminase (GPT) compared to controls, but these levels were not significant($P<0.05$). Treatment did not have a significant difference on these levels in diabetic rats. However, extract treated normal controls had lower GOT and GPT levels compared to untreated normal controls. These results are shown in Figure 17 below.

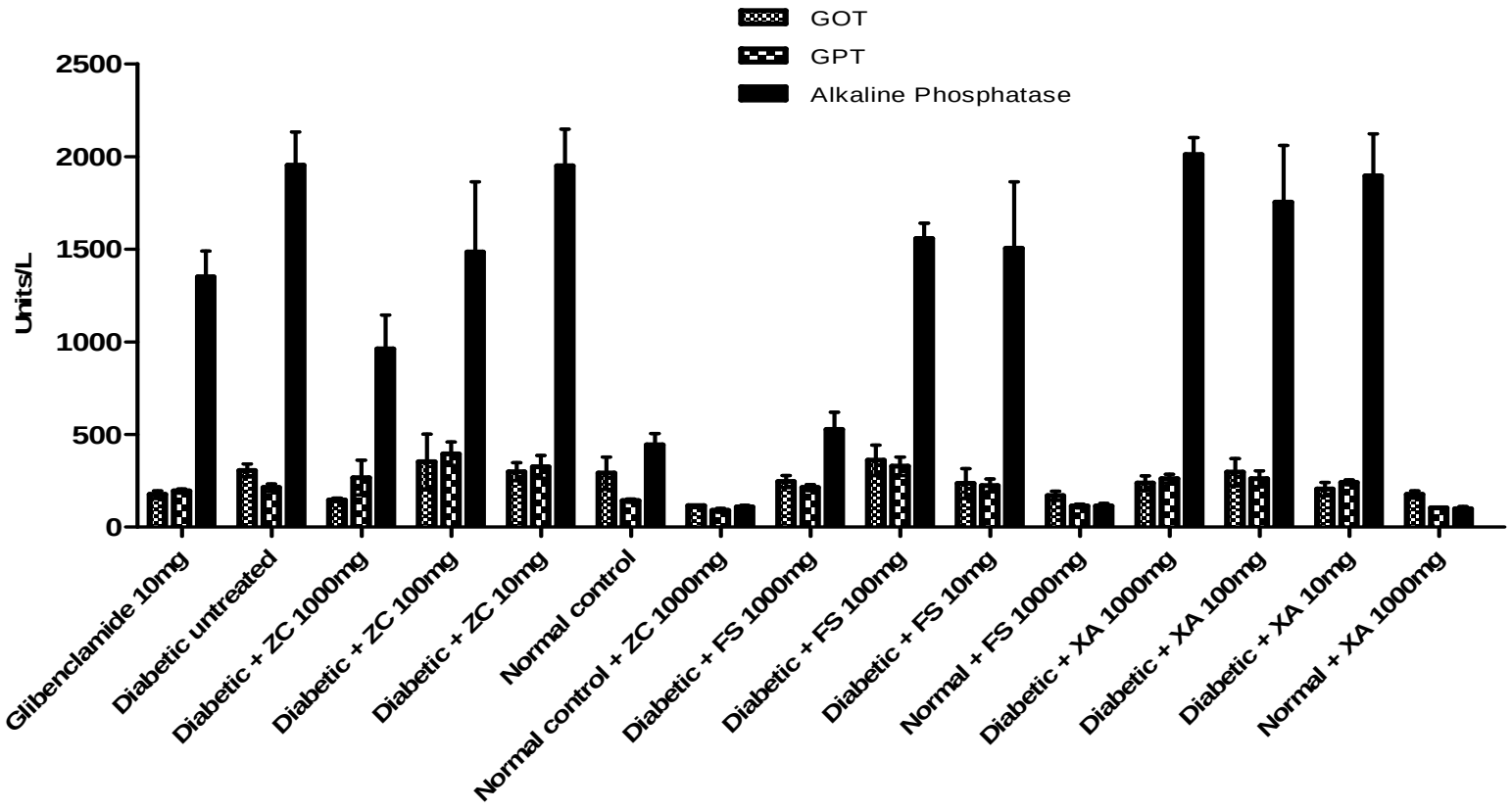


Figure 17: Effect of *Z. chalybeum*, *F. sycomorus* and *X. americana* stem bark extracts on hepatic enzymes (GOT, GPT and alkaline phosphatase) in normal and diabetic rats

4.10 Effect *Z. chalybeum*, *F. sycomorus* and *X. americana* stem bark extracts on serum creatinine and bilirubin

Extract treated normal controls had lower creatinine levels compared to untreated normal rats although the difference was not statistically significant. Extract treated normal rats had higher bilirubin levels compared to untreated normal controls and diabetic rats. These levels were however not significantly different. Creatinine and bilirubin levels were not significantly different in diabetic rats within the various treatment groups ($P<0.05$). Results are expressed in Figure 18 below as mg/dl.

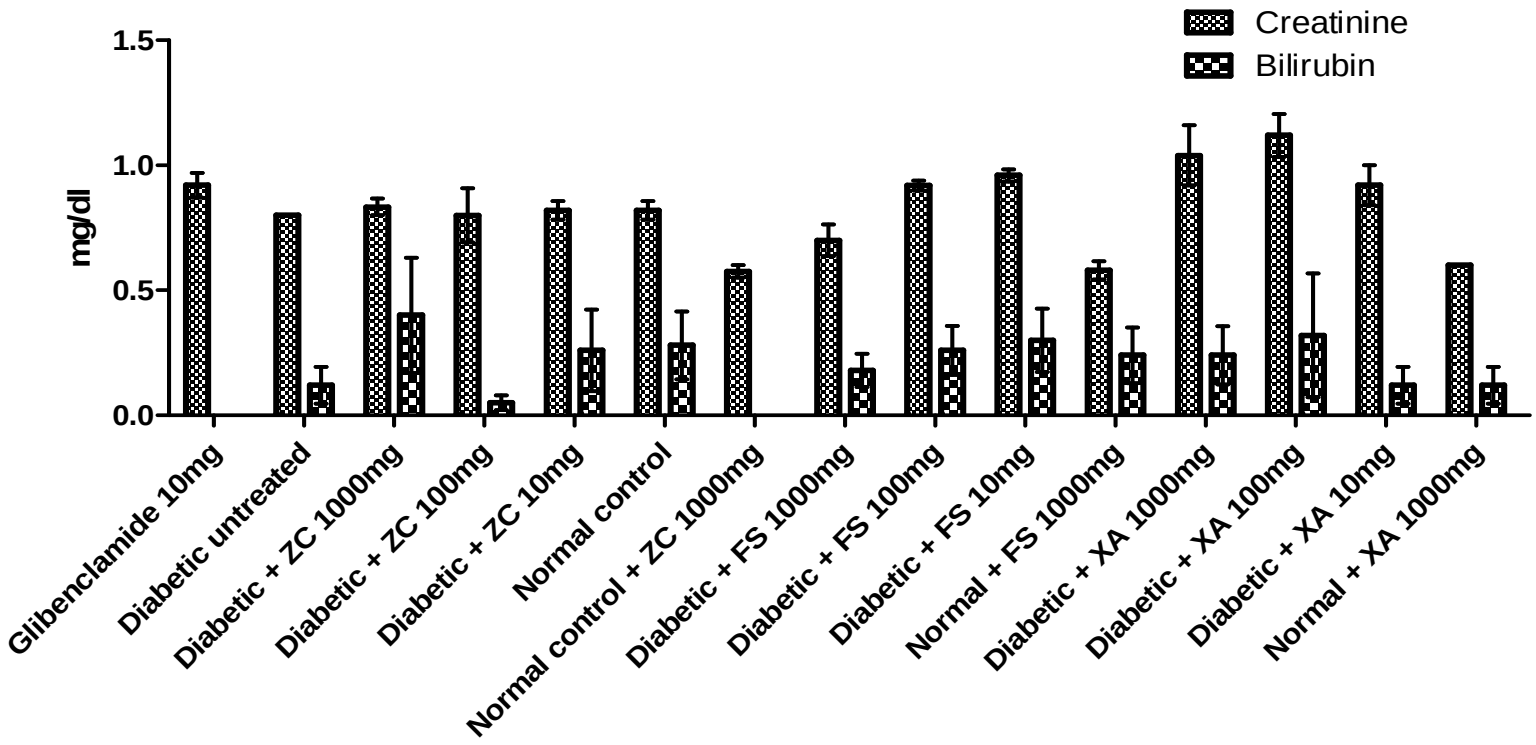


Figure 18: Effect of *Z. chalybeum*, *F. sycomorus* and *X. americana* stem bark extracts on serum creatinine and bilirubin levels in normal and diabetic rats

4.11 Effect *Z. chalybeum*, *F. sycomorus* and *X. americana* stem bark extracts on serum urea

Extract treated normal controls had lower urea levels compared to untreated normal controls but these levels were not significantly different. Serum urea levels were not significantly different between the different diabetic treatment groups ($P<0.05$). Results are presented in Figure 19 below as mg/dl.

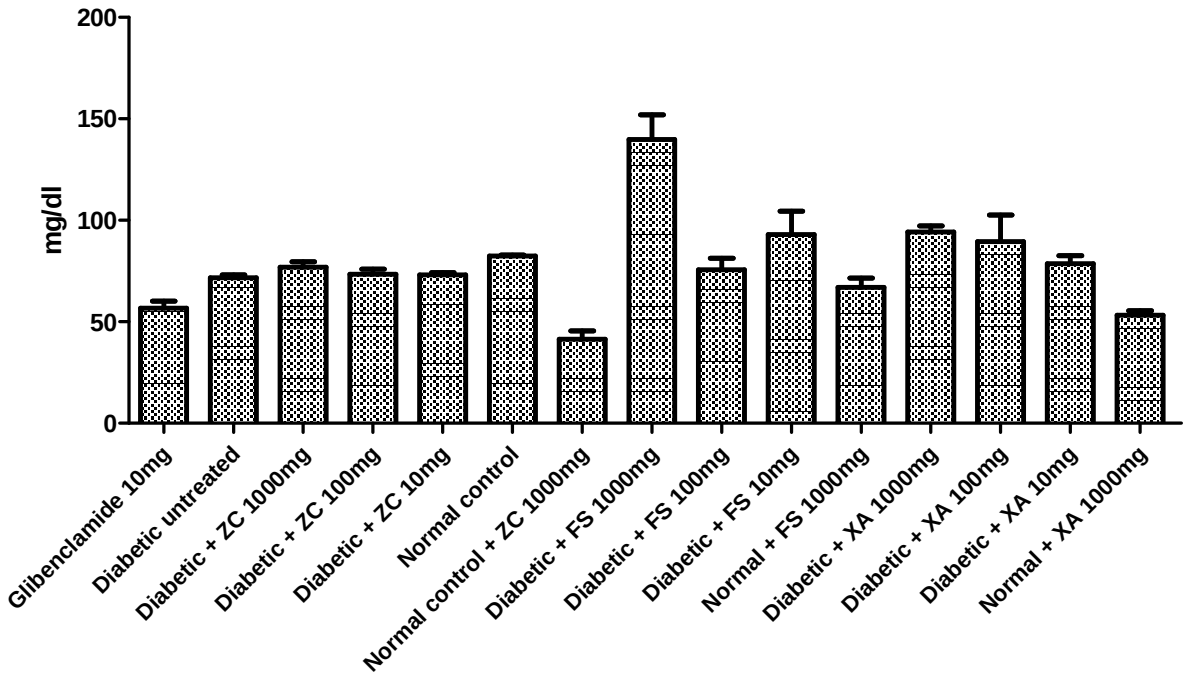


Figure 19: Effect of *Z. chalybeum*, *F. sycomorus* and *X. americana* stem bark extracts on serum urea levels in normal and diabetic rats

4.12 Effect *Z. chalybeum*, *F. sycomorus* and *X. americana* stem bark extracts on serum total protein and albumin

There was no significant difference between levels of total protein and albumin in serum across different extract treatments and diabetic and normal control groups. These results were expressed as g/dl as shown in Figure 20 below.

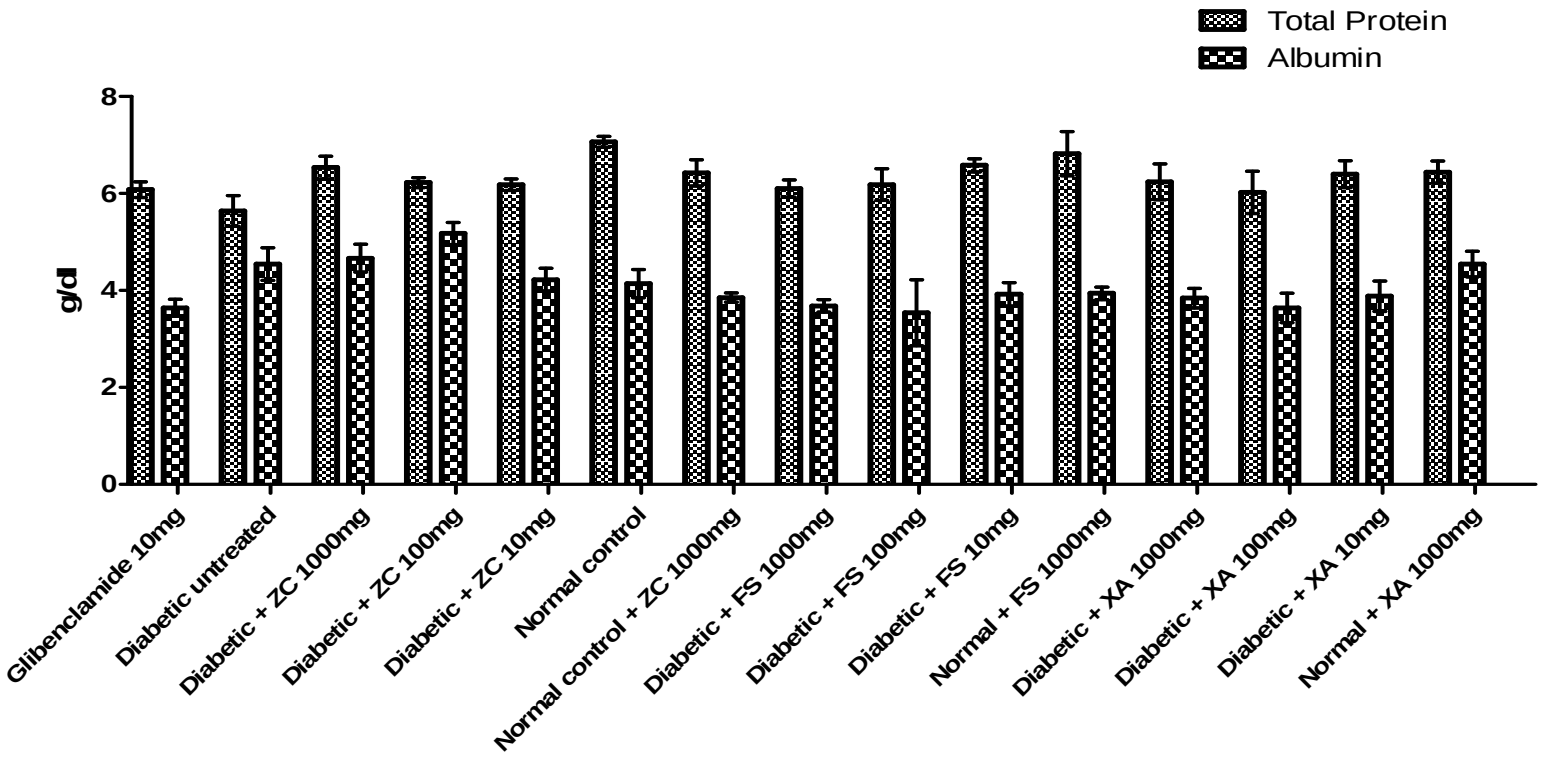
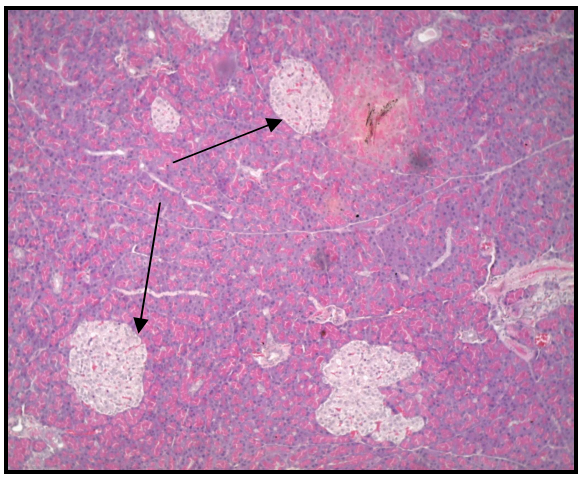


Figure 20: Effect of *Z. chalybeum*, *F. sycomorus* and *X. americana* stem bark extracts on serum total protein and albumin in normal and diabetic rats

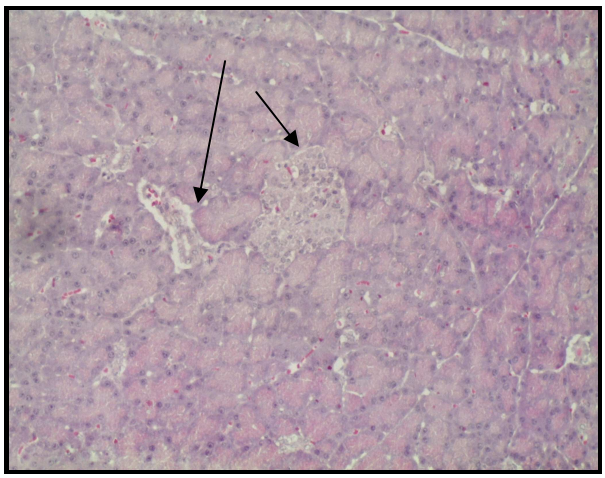
4.13 Histological findings

4.13.1 Effect of *Z. chalybeum*, *F. sycomorus* and *X. americana* stem bark extracts on the histology of the pancreas

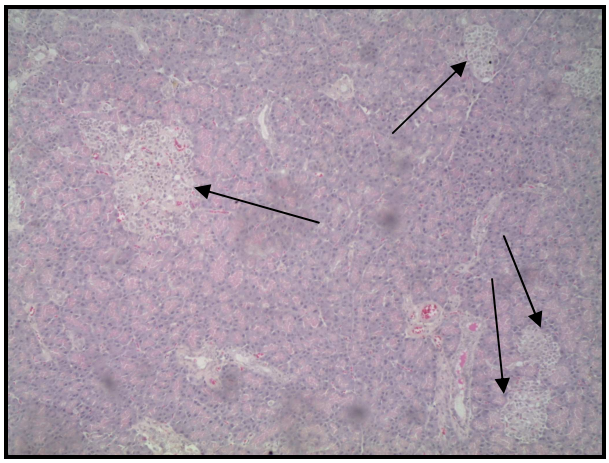
Administration of STZ decreased the number of β - cells and the sections from untreated diabetic rats demonstrated shrunken islets of Langerhans with degenerative necrosis. In the sections from extract treated rats, the islets of Langerhans appeared less shrunken compared to those from the untreated group and were also more in number (Figure 21). All three extracts showed a higher number of normal islets of Langerhans compared to untreated diabetic rats.



(A) Normal control showing normal Islet (arrows) morphology, size and number (Magnification 10x)



(B) Section from an untreated diabetic rat showing abnormal Islets with fewer islets compared to the normal controls (Magnification 10x)

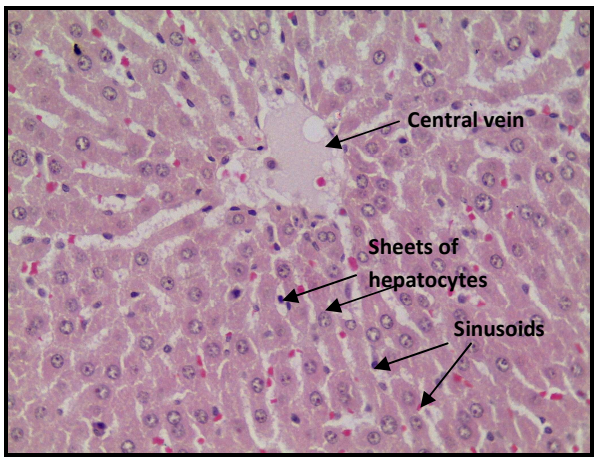


(C) Extract treated diabetic rat pancreas showing increased number of islets (arrows) compared to untreated diabetic rats (Magnification 10x)

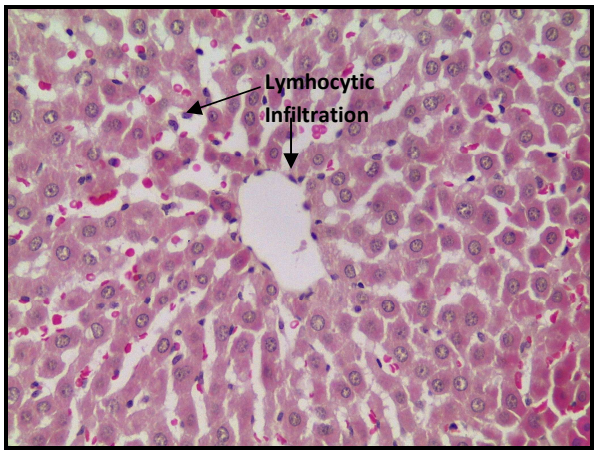
Figure 21: Effect of *Z. chalybeum*, *F. sycomorus* and *X. americana* stem bark extracts on the histology of the pancreas in normal and diabetic rats

4.13.2 Effect of *Z. chalybeum*, *F. sycomorus* and *X. americana* on the histology of the liver

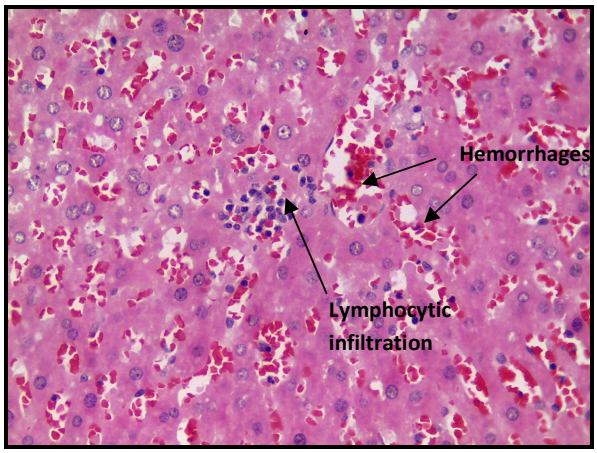
Diabetic rat livers exhibited loss of normal architecture, narrowing of sinusoids, infiltration by lymphocytic cells, hepatocyte degeneration and hemorrhage. Figure 22 shows normal liver histology in the normal rats, and moderate and severe pathology in the diabetic rats. Extract treated rats demonstrated normalization of liver histology with normal architecture, decreased hemorrhages with presence of little or no infiltration by lymphocytic cells.



(A) Liver in normal control rats showing normal architecture (Magnification 10x)



(B) Liver in extract treated rats diabetic rats showing mild hemorrhages and mild lymphocytic infiltration (Magnification 10x)

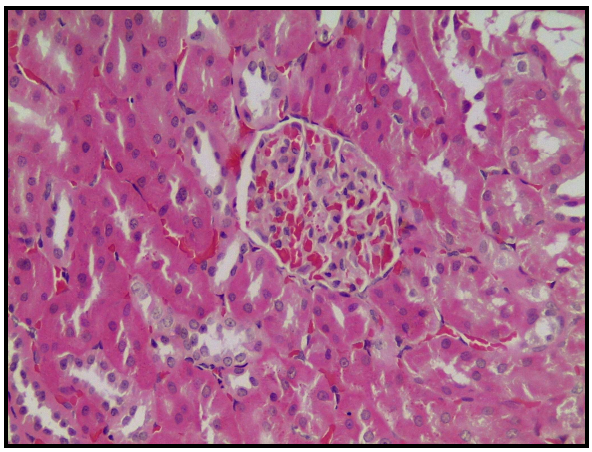


(C) Liver in untreated diabetic rats showing abnormal cellular architecture, hemorrhages and lymphocyte infiltration (Magnification 10x)

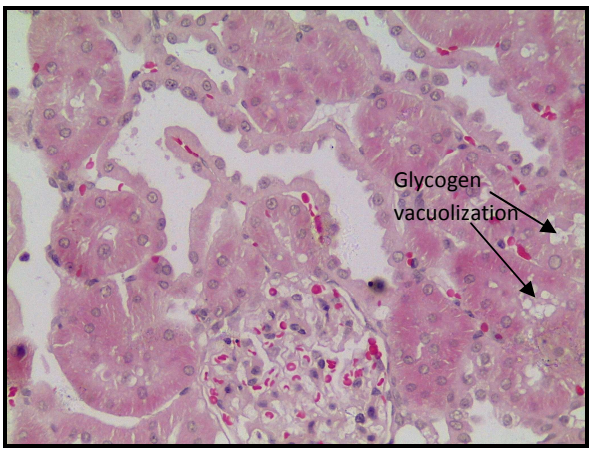
Figure 22: Effect of *Z. chalybeum*, *F. sycomorus* and *X. americana* stem bark extracts on the histology of the liver in normal and diabetic rats

4.13.3 Effect of *Z. chalybeum*, *F. sycomorus* and *X. americana* on the histology of the kidneys

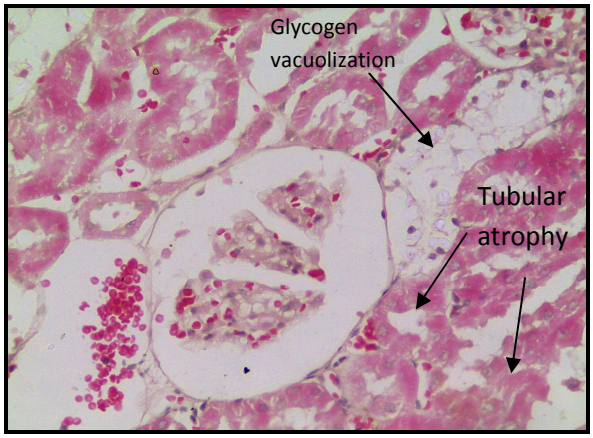
Diabetic rats showed kidney pathology with glomerulosclerosis, hyalinization of the blood vessel walls, tubular atrophy and glycogen vacuolization of renal tubular epithelial cells. Additionally, there was thickening of tubular basement membranes, lymphocytic infiltration in interstitial spaces, loss of brush border in tubular epithelial cells, loss of architecture and rapture of cell membranes. There was no difference between extract treated and non-treated diabetic rats. Results are summarized in Figure 23 below.



(A) Kidney in normal rats showing normal Architecture (Magnification 40x)



(B) Kidney section from a diabetic rat showing glycogen vacuolization and loss of celluar architecture (Magnification 40x)



(C) Kidney in diabetic rats showing loss of architecture and rapture of cell membranes (Magnification 40x)

Figure 23: Effect of *Z. chalybeum*, *F. sycomorus* and *X. americana* stem bark extracts on the histology of the kidneys

CHAPTER FIVE

DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 DISCUSSION

Data obtained from the informants shows that traditional knowledge on medicinal plants and plant use is prevalent in the studied region. The rational use of herbal medicine products requires that adverse effects and potential interactions are recorded. The establishment of pharmacovigilance programs for herbal products is thus important. The World Health Organization has issued guidelines addressing this issue (WHO Expert Committee on Diabetes, 1980).

In the present study, *Z. chalybeum* extract was observed to have significant antidiabetic effects in streptozotocin- induced diabetic rats compared to untreated diabetic controls. Daily administration of varying concentrations of *Z. chalybeum* extract to diabetic rats for 2 weeks produced a dose-dependent reduction in fasting blood glucose levels. This decrease in fasting blood glucose extract treated diabetic rats was not significantly different from that of diabetic rats treated with Glibenclamide at 10mg/kg body weight. Diabetic rats administered 1000mg and 100mg/kg bwt of the extract also had fasting blood glucose levels that were not significantly different from those of the normal controls ($P<0.05$). This is the first report of the efficacy of *Z. chalybeum* against diabetes mellitus in an experimental setting. Other species in the genus *Zanthoxylum* have however been studied experimentally, with significant antidiabetic activity reported. For example, various parts of *Z. zanthoxyloides* including the roots, bark and leaves have been used for medicinal purposes, including the treatment of diabetes mellitus.

Significantly ($P < 0.05$) lower blood glucose was observed in the treated animals in comparison to non-treated groups (Aloke *et al*., 2012). Other species in the genus that are used traditionally to treat diabetes are *Z. armatum* is used in Nepal, and *Z. nitidum* in India (Singh and Singh, 2011; Arun and Paridhavi, 2012). The beneficial effect of *Z. chalybeum* treatment in diabetic rats was likely due to improved insulin release and glucose uptake in remnant β-cells (Buchanan, 2003). Increased insulin secretion following *Z. chalybeum* could also increase conversion of blood glucose into glycogen by enhancing the glycolytic and glycogenic processes with concomitant decrease in glycogenolysis and gluconeogenesis (Arya *et al.*, 2012). The hypoglycemic activity of *Z. chalybeum* observed in this study may be attributed to the secondary metabolites identified through phytochemical screening. These include alkaloids, saponins, glycosides, tannins, terpenoids and phenols. In general, there is very little biological knowledge on the specific modes of action in the treatment of diabetes, but most of the plants have been found to contain substances like glycosides, alkaloids, terpenoids and flavonoids that are frequently implicated as having antidiabetic effects (Loew and Kaszkin, 2002). These phytochemicals possess wide therapeutic benefits and studies have demonstrated anti-diabetic, anti-oxidant, and anti-inflammatory activities with these compounds (Piero *et al.*, 2011; Arya *et al.*, 2012; Shih *et al*., 2012). Medicinal properties of this genus have been attributed to the presence of secondary metabolites like alkaloids, sterols, flavonoids, aliphatic and aromatic amides, lignins, coumarins, sterols, carbohydrate residues (Aloke *et al*., 2012). Thus, combination of these compounds in *Z. chalybeum* may exert synergistic anti-diabetic effects in the diabetic rats.

F. sycomorus significantly reduced fasting blood glucose levels at doses of 100mg and 10mg/kg body weight compared to untreated diabetic rats. The higher dose, 1000mg/kg body weight was not statistically significant. The results were similar when compared with the Glibenclamide treated rats and normal controls where the 100mg and 10mg/kg body weight groups showed no significant difference while the 1000mg/kg body weight group was significantly higher. These results concur with those of Aduom *et al* (2012) who reported significant hypoglycemic activity in diabetic rats treated intraperitoneally with 250mg/kg body weight of the methanolic extract of *F. sycomorus* stem bark compared with untreated diabetic rats ($P<0.05$). In this study too, the hypoglycemic effect of the methanolic stem bark extract was not dose dependent. This could be due to antagonism. The extract contained many secondary metabolites, some of which could be antagonistic. Therefore, at low doses, the concentration of these antagonistic molecules was low and thus, offering no hindrance to the antidiabetic substances present in the extract. A similar observation was reported on the hypoglycaemic effect of bark extract of *Pterocarpus santalinus* on blood glucose concentration in streptozotocin-induced diabetic rats (Aduom *et al*., 2012). The aqueous stem bark extract injected intraperitoneally, was found to lower blood glucose as effectively as insulin 3 hours after treatment at doses of 50 mg/kg body weight, 100mg/kg body weight and 150 mg/kg body weight in alloxan induced diabetic rats (Njagi *et al*., 2012). Other species in the genus *Ficus* have also been studied for their antidiabetic activity. The, alcoholic extract of *F. bengalensis* stem bark at a dose of 25mg, 50mg and 75mg/day/100g, body weight lowered the blood glucose level 47 to 70%, and also restored the normal levels of serum urea, cholesterol and total protein of alloxan diabetic albino rats (Gupta *et al*., 2008). Continuous

treatment of STZ-treated SHR and obese Zucker diabetic rats with ethanolic extract of *F. exasperata* (FEE) for a period of 4 weeks caused significant decrease ($p<0.05$) in blood glucose levels of the FEE treated diabetic rats (Adewole *et al*., 2011). *Ficus retusa* L. "variegata", alcoholic extract (400 mg/kg) was found to reduce blood glucose levels of diabetic rats significantly compared to the diabetic group (Sarg *et al*., 2011).The ethanolic extract of leaves of *F. glomerata* had significant antihyperglycemic effect in the experimental albino rat model of diabetes mellitus. *F. exasperate* Vahl and *F. arnottiana* Miq. are also reported to have antidiabetic activity (Khan *et al*., 2012; Dhungana *et al*., 2013). The antidiabetic activity of various *Ficus* spp. is postulated to be due to the presence of various chemical compounds including, alkaloids, flavonoids, saponins, tannins, glycosides, gallic and reducing sugars. Additionally, elements including potassium, calcium, chromium, manganese, iron, copper and zinc which are responsible for initiating insulin function have been shown to be present, but the levels differ with the plant part and species (Khan *et al*., 2012).

X. americana administered at 10mg and 100mg and 1000mg/kg body weight reduced fasting blood glucose significantly compared to untreated diabetic rats. At all three dose levels; 10mg, 100mg and 1000mg/kg body weight fasting blood glucose levels that were not significantly different compared to the Glibenclamide treated rats. Siddaiah *et al* (2011) showed that the methanolic extract *X. americana* leaves had a significant dose dependent hypoglycemic effect in alloxan induced diabetic rats at doses of 200, 400 and 600mg/kg bwt ($P<0.05$). The observed hypoglycemic activity of *X. americana* stem bark extract may be due to the presence of

secondary metabolites including saponins, glycosides, flavonoids, tannins, phenolics, alkaloids, quinones and terpenoids. Studies have isolated saponins, glycosides, flavonoids, tannins, phenolics, alkaloids, quinones and terpenoids from of crude *X. americana* aqueous, methanolic, ethanolic, butanolic and chloroform extracts from leaves, roots and stem bark (Siddaiah *et al*., 2011; Monte *et al*., 2012). Flavonoids are known to be used for the treatment of diabetes thus the presence of flavonoids and tannins may have been responsible for the observed antidiabetic activity (Siddaiah *et al*., 2011).

The anti-hyperglycemic action of the extracts observed may result from potentiating the insulin effect of plasma by stimulating insulin release from the remnant pancreatic β-cells or its release from the bound form. Additionally, it might involve extra-pancreatic action in these including the stimulation of peripheral glucose utilization or enhancing glycolytic and glycogenic processes with concomitant decrease in glycogenolysis and gluconeogenesis (Pareek *et al.*, 2009).

Following administration of 2g/kg body weight glucose orally, all three extracts resulted in a gradual decrease in blood glucose levels which was dose dependent for *Z. chalybeum* extract, and not for *F. sycomorus* and *X. americana*. There was no significant difference in glucose tolerance between extract treated normal rats and untreated normal controls ($P<0.05$). This may indicate that the effects of the extracts in lowering glucose are not acute but long term. These results suggest that the extracts could not directly stimulate insulin secretion or insulin

sensitivity. A study indicated that there was no significant improvement in insulin level in diabetic rats supplemented for 2 weeks with *Inula viscose* L. a medicinal plant commonly used in Morocco for treatment of diabetes (Abbe *et al*., 2004). Moreover, in another study there was no significant improvement in insulin level in diabetic patients supplemented with psyllium seeds from *Plantago ovata* Forsk. Therefore it could be suggested that the hypoglycaemic properties of the extracts were not solely dependent on insulin action or secretion (Abbe *et al*., 2004). The different constituents of antidiabetic plants could have different sites of action in the body (Jarald *et al*., 2008). Other possible mechanisms of antidiabetic plants are adrenomimeticism, pancreatic beta cell potassium channel blocking, cAMP (2nd messenger) stimulation (Marles and Farnsworth, 1996), inhibition in renal glucose reabsorption (Eddouks *et al*., 2002), inhibition of insulin degradative processes and reduction in insulin resistance (Pulok *et al*., 2006), providing certain necessary elements like calcium, zinc, magnesium, manganese and copper for the beta-cells (Mohamed *et al*., 2006), regenerating and/or repairing pancreatic beta cells (Mohamed *et al*., 2006), increasing the size and number of cells in the islets of Langerhans (Mohamed *et al*., 2006), stimulation of insulin secretion (Esmaeili and Yazdanparast, 2004), stimulation of glycogenesis and hepatic glycolysis (Miura *et al*., 2001), protective effect on the destruction of the beta cells (Kim *et al*., 2003), improvement in digestion along with reduction in blood sugar and urea (Krishnan, 1968), prevention of pathological conversion of starch to glucose (Sepha and Bose, 1956), inhibition of β-galactocidase and α–glucocidase (Sharma and Mujumdar, 1990), cortisol lowering activities (Gholap and Kar, 2004), inhibition of alpha-amylase (Heidari

et al., 2005) and preventing oxidative stress that is possibly involved in pancreatic β-cell dysfunction found in diabetes (Hideaki *et al*., 2005).

There was significant loss in body weight of diabetic rats compared to normal rats, a symptom synonymous with diabetes mellitus. The loss of body weight associated with STZ-induced diabetes could be due to dehydration and catabolism of fats or breakdown of tissue proteins, with consequent wasting of muscle (Pupim *et al*., 2005). Normal body weight gain is indicator of efficient glucose homeostasis; but in diabetics, glucose is not available therefore the cells use alternatively proteins for energy; consequently due to excessive breakdown of tissue protein a loss in body weight occurs. Treatment with *Z. chalybeum* resulted in a reduction in body weight loss compared to untreated diabetic rats ($P<0.05$). This can be attributed to the improvement in glycemic control. Similar effect on body weight gain was previously reported with other plants, well known for their anti-diabetic activity (Pareek *et al*., 2009). This is also in agreement with the finding that normal controls that were given the three extracts had a higher weight gain compared to the untreated normal controls. This could in part be explained also by the fact that extract treated normal controls had a higher food intake compared to untreated normal controls.

F. sycomorus treated diabetic rats at 100mg/kg bwt and 10mg/kg bwt had a similar weight loss to the untreated diabetic rats. However, the higher dose 1000mg/kg bwt reduced the weight loss in diabetic rats to levels that were not different from those of the Glibenclamide treated diabetic rats. Diabetic rats that were given *X. americana* extract showed a dose dependent decrease in

body weight, which was greater at lower doses of the extract. For all extract treated diabetic groups, the rats were unable to recover body weights to pre-diabetic levels. Other studies have also reported the same trend using other antidiabetic plant extracts (Ahmed and Urooj, 2008). This could be as a result of the severity of the streptozotocin induced hyperglycemia resulting in minimal residual functional pancreatic β-cells (Keller *et al*., 2009).

Diabetic rats had a significantly higher food intake compared to normal controls. This was not remedied by treatment either with extract or Glibenclamide. Polyphagia is a classic symptom of diabetic mellitus resulting from abnormalities in carbohydrate metabolism. This is caused by the inadequate levels of insulin in the body. Glucose entry into cells is dependent on insulin and therefore without insulin the cells cannot take up glucose from the blood stream and they effectively start to starve. The body's response to cellular starvation is to increase the concentration of glucose and ketone bodies in blood. However, without insulin the cells cannot use the glucose or ketone bodies and they therefore continue to starve. In diabetes due to insulin resistance or absence of insulin, glucose cannot move into the satiety center thus the arteriovenous difference remains low and the feeding center is chronically active (Gerich, 2000; DeFronzo, 2004; Guyton and Hall, 2006).

The diabetic rats were observed to have significantly higher water intake compared to the normal controls. Treatment with extract or Glibenclamide did not reduce these levels back to normal. The increased water intake is a result of prolonged hyperglycemia. The kidneys cannot handle

such a high level of glucose and they start to leak massive amounts of glucose into urine. As the kidneys cannot excrete glucose without water, large amounts are passed osmotically into the renal tubules and excreted along with the excessive glucose. The result is excessive intake of water to prevent dehydration and the consequent excessive passing of urine (Gerich, 2000; DeFronzo, 2004; Guyton and Hall, 2006). Ahmed and Urooj (2008) also reported significantly increased water intake in *F. glomerata* extract treated diabetic rats compared to normal controls.

Diabetes mellitus is associated with high levels of circulatory cholesterol and other lipids and this accounts for the atherosclerosis, arteriosclerosis and severe coronary heart disease which leads to increase in levels of transaminases, marker enzymes important in heart and liver damage. Studies have observed that the liver is necrotized in diabetic patients. Therefore, the increment of the activities of GOT, GPT, and ALP in plasma may be mainly due to the leakage of these enzymes from the liver cytosol into the blood stream, as a result of the hepatotoxic effect of STZ (El-Demerdash *et al*., 2005). Other studies have also shown that STZ results in a significant increase in serum levels of GOT, GPT and ALP 1, 3 and 6 hours after treatment (Ragbetli and Ceylan, 2010). The study established that the level of increase of these enzymes was dependent on the dose of STZ administered with higher STZ doses resulting in a greater increase in enzyme levels. Ragbetli and Ceylan (2010) also report that studies are needed to verify and clarify the relationship between different doses streptozotocin induced diabetes and biochemical parameters. The levels of GOT, GPT and ALP have been reported to be increased in alloxan-induced diabetic rats. In this study *Z. chalybeum* and *F. sycomorus* treated diabetic rats

had significantly ($P<0.05$) reduced levels of ALP thus improving renal and hepatic functions. This observation is consistent with earlier reports on hepatoprotective potentials of leaf extracts of *V. amygdalina* in mice (Akah *et al.,* 2009). Treatment of diabetic rats with either onion or garlic was observed to cause a reduction in the activity of these enzymes in plasma compared to the untreated diabetic group (El-Demerdash *et al*., 2005). Rawi *et al* (2011) observed a highly significant decrease in activity of serum and liver ALP after four weeks of continous treatment of diabetic rats with either glibenclamide, *M. indica, P. guajava* or the mixture of both *M. indica, P. guajava* as compared with diabetic control ($P<0.05$). The slightly elevated levels of GOT and GPT in diabetic rats compared to normal controls may be the result of the greater need for gluconeogenic substrate in the diabetic rats. The elevation of both enzymes may also reflect damage of the hepatic cells (Rawi *et al.,* 2011). Treatment with the extracts and Glibenclamide in diabetic rats did not result in a significant decrease in the levels of these enzymes. The effect of the extract in lowering GOT and GPT levels was however observed in extract treated normal rats which were lower compared to untreated normal controls. This decrease in serum GOT and GPT may be attributed to the presence of tannins and flavonoids in the plant extracts (Rawi *et al*., 2011). The significant decrease in serum ALP activity indicates the protective effect of the extracts on the liver and improvement in liver function (Rawi *et al*., 2011). Serum levels of urea and creatinine in diabetic rats were not significantly different from those of normal rats. Elevated serum levels of urea and creatinine are significant markers of renal dysfunction. These results thus indicate that diabetic rats did not suffer any renal dysfunction (El-Demerdash *et al*., 2005).

In the histological analysis of the pancreas, the islets of Langerhans from extract treated rats appeared less shrunken compared to those from the untreated group and were also more in number. The improvement in pathology in extract treated rats was similar to that of diabetic rats that were given Glibenclamide. These results concur with those of Koshy *et al* (2012) who reported that *Elytraria acaulis* was able to reduce the pancreatic damage in streptozotocin induced diabetic rats. Rats treated with Glibenclamide showed diffused necrotic changes of mild to moderate degree in the pancreas with a mild reduction in the size and number of the islets in this group. The effect of *E. acaulis* extract (400 mg/kg) on streptozotocin diabetic rats was comparable with that of Glibenclamide. The pancreatic damage observed in Glibenclamide and *E. acaulis* extract treated diabetic animals was milder than that in the untreated diabetic control group. *Costus pictus* was shown to increase the area and diameter of pancreatic islets in streptozotocin induced diabetic rats compared to untreated diabetic controls (Jayasri *et al*., 2008). Islet cells of diabetic rats treated with 500mg/kg/day of *Alchornea cordifolia* plant extract regenerated considerably suggesting the presence of stable cells in the islets with the ability of regenerating (De Fronzo *et al*., 1997) suggesting that the plant extract at this dose could induce the quiescent cells to proliferate to replace the lost cells. The exact mechanism is not known but it has been documented that the flavonoid fraction of this plant extract decreases blood glucose and increases the number of β-cells (Chakravarthy *et al.*, 1980). It has also been documented that phenolic content of therapeutic plants contributes immensely to their antioxidant activity. The phenolic constituent may have stopped further destruction of the remaining β-cells in the islet by mopping up the circulating reactive oxygen species generated by the alloxan to destroy the β-

cells and then allowing other phytochemicals of the plant to induce regenerative activities (Ikechukwu and Obri, 2009). Other studies also support the protective effect of antioxidant defense mechanisms against streptozotocin induced pancreatic damage. Anthocyanins were found to protect pancreatic tissue including islet β-cells against apoptosis induced by streptozotocin through the regulation of apoptosis and in this way prevent loss of islet viability and functionality. They were also found to restore antioxidant-defense mechanisms, thus protecting tissues from oxidative damage in the diabetic state (Nizamutdinova *et al.,* 2009). *Costus pictus* aqueous extract was found to have strong antioxidant activity. This may be due to the presence of phenols and flavonoids, which may have a major role in reducing oxidative stress associated with diabetes (Jayasri *et al*., 2008). Methanol extracts of *Moringa oleifera* pods were effective in preventing oxidative protein damage, which is thought to be involved in β-cell damage in streptozotocin induced diabetic rats bringing about regeneration of pancreatic β-cells (Gupta *et al*., 2012). This may be true for the aqueous extracts of *Z. chalybeum*, *F. sycomorus* and *X. americana* which were found to contain flavonoids and phenols. The histological findings are in concordance with improved glycemia observed in streptozotocin induced diabetic rats that were given extracts of *Z. chalybeum*, *F. sycomorus* and *X. americana*. Oxidative stress has been shown to play a role in the pathogenesis of diabetes consequently; antioxidants may have a role in the alleviation of diabetes. Streptozotocin produces oxygen radicals in the body, which cause pancreatic injury leading to hyperglycemia (John, 1991).

In the liver, extract treated rats demonstrated normalization of liver histology with normal architecture, decreased hemorrhages with presence of little or no infiltration by lymphocytic cells. Other studies have shown similar results with other extracts. *Mormodica charantia* fruit aqueous extract had ameliorative effects in streptozotocin induced hepatic damage (Abdollahi *et al*., 2010). In another study, curcumin treated diabetic rats showed improved liver pathology when compared to diabetic controls (Soetikno *et al*., 2012). Diabetes-induced liver injury was associated with increased amounts of lipid peroxidation and decreased antioxidant enzyme, indicating oxidative stress, and the dephosphorylation of adenosine monophosphate-activated protein kinase (AMPK) as well as translocation of protein kinase C (PKC-α) to the membrane in the liver of rats with streptozotocin-induced diabetes. Moreover, in STZ-induced diabetic rats, there were excessive amounts of lipid deposits in the liver sections, as shown by hematoxylin and eosin staining; all of these abnormalities were ameliorated by curcumin treatment. Curcumin treatment also significantly suppressed NF-κB activity as well as reduced the degradation of cytosolic IκBα: as a consequence, the level of proinflammatory cytokines, TNF-α and IL-1β were further significantly decreased. Curcumin treatment markedly inhibited diabetes-induced increased expression of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase subunits (p67*phox* and p22*phox*), nitrotyrosine and inducible nitrous oxide synthase (iNOS) which is an essential mechanism responsible for increased reactive oxygen species production (Soetikno *et al*., 2012). Zafar *et al* (2009a) showed a progressive development of the lesions in the liver of diabetic rats which seemed to be due to streptozotocin. Most liver sections showed increased fibrosis with plasmacytic infiltrate causing distortion of the usual concentric arrangement of

hepatocytes. There was also congestion of portal vessels and sinusoids and the veins were also dilated. Their study showed that GOT and GPT levels were significantly increased in the serum of streptozotocin-treated animals, thus corroborating results from this study. The increase in serum aminotransferases levels may be due to the cellular damage in the liver caused by streptozotocin-induced diabetes. The detailed mechanism by which enzymes are released from the cytosol and mitochondria of hepatocytes is not completely known (Zafar *et al*., 2009a). Experimental studies have shown that subtle membrane changes are sufficient to allow passage of intracellular enzymes to the extracellular space (Garella, 1997). A very large concentration gradient between the hepatocytes and the sinusoidal space usually exists for enzymes. Cell damage increases permeability causing cytosolic isoenzymes to spill into the sinusoids and from there into the peripheral blood (Garella, 1997). It has been shown by Rogers *et al* (1986), that mitochondrial activity was decreased 53 % per gram of diabetic liver and cytoplasmic GOT activity was increased 3-4 fold in STZ diabetic rats. Voss *et al* (1988) proposed that STZ in hyperglycemic animals caused a time dependent rise in GOT, GPT, and ALP levels. The work of Barneo *et al*. (1990) showed that STZ-induced diabetes in rats produced alterations in hepatic functions as described in poorly controlled diabetics. This alteration in hepatic function may be because of increased activity and mRNA levels of araginase as reported by Salimuddin *et al* (2008) in their study. Hepatocellular dysfunction was evaluated by the GOT and GPT activities in plasma. The results of this study showed that streptozotocin diabetes in rats produced alterations in the hepatic functions as well as structure of hepatocytes. The increase in the levels of GOT and GPT in diabetic rats after 1-3 weeks treatment was also reported by many other

workers (Zhang *et al*., 1995; Isogai *et al*., 1997). Okada *et al* (1997) reported that GOT activity was lower than the amount of enzyme in diabetic rat tissues. Alkaline phosphatase is a membrane bound glycoprotein enzyme. It is present in highest concentrations in the sinusoids and in the endothelium of the central and periportal veins; smaller concentrations occur in the biliary canaliculi. Barneo *et al.* (1990) evaluated cholestasis by plasma ALP activity in STZ induced diabetes and their results showed that ALP levels were raised. Leibovitch *et al* (1991) observed increased levels of serum ALP in pathological conditions involving the kidneys and liver. Increase in the levels of ALP in diabetic rats was also reported by Ramesh and Pugalendi (2006). These results corroborate findings from this study. *Annona squamosa* L. aqueous leaf extract ameliorated liver tissue damage in a dose dependent manner in which hepatocytes possessed regular size of nuclei, well defined cell boundaries, reduced vacuoles and granulated characters as well as narrowed sinusoids when compared with those of control diabetic rats (Rabintossaporn *et al*., 2009). The improvement of histological morphology of liver in diabetic rats that received aqueous leaf extract of *A. squamosa* is probably explained by its hypoglycemic effect caused from anti-oxidant activities of its flavonoids (Shirwaikar *et al*., 2004), thus decreasing of β-cell injury and reducing hyperglycema. The mucilage obtained from the extract may absorb the blood glucose (Riyad *et al*., 1988; Ajabnoor *et al*., 1990) and unknown active ingredients stimulate either the pancreatic insulin secretion from the existing β-cells or its release from the bound form (Shirwaikar *et al*., 2004). These mechanisms may synergize to restore glucose metabolism, lead to the recovery of normal metabolic process. In addition to the liver histological improvement caused by hypoglycemic effect, the anti-oxidant properties of the

extract itself may also directly improve the histological features of the liver by free radical scavenging ability (Saija *et al*., 1995; Shirwaikar *et al*., 2004; Rabintossaporn *et al*., 2009).

Administration of streptozotocin resulted in degenerative changes in the kidneys of diabetic rats characterized by glomerulosclerosis, hyalinization of blood vessel walls, tubular atrophy, glycogen vacuolization of renal tubular epithelial cells, thickening of tubular basement membranes, lymphocytic infiltration in interstitial spaces, loss of brush border in tubular epithelial cells, loss of architecture and rapture of cell membranes. Similar findings have been reported in other studies (Zafer *et al*., 2009b; Teoh *et al*., 2010; Koshy *et al*., 2012). Treatment with extract did not improve the histological changes observed. This is in contrast to other studies that have shown amelioration of renal damage after administration of plant extracts (Tedong *et al*., 2006; Teoh *et al*., 2010; Ganesh *et al*., 2012). This might be due to the extent of renal damage in this case or the fact that higher doses of the extracts might have been required. Studies have shown that this process of renal repair can be dose dependent (Koshy *et al*., 2012; Basha and Saumya, 2013).

5.2 CONCLUSIONS

The use of traditional medicine for the treatment of diabetes mellitus is prevalent in the area studied. With the knowledge being passed orally down generations. There is a need therefore to document this knowledge for posterity as well as to enable a systematic classification and investigation of the medicinal uses and antidiabetic activity and toxicity of these plants. This will

also ensure sustainable use of these natural resources to prevent depletion or extinction of useful species. The following conclusions were made based on results obtained.

1. *Zanthoxylum chalybeum* stem bark extract resulted in a dose dependent decrease in fasting blood glucose levels in diabetic rats that was not significantly different from that of Glibenclamide at 10mg/kg body weight. Diabetic rats administered 1000mg and 100mg/kg body weight of the extract also had fasting blood glucose levels that were not significantly different from those of the normal controls ($P<0.05$).
2. *F. sycomorus* significantly reduced fasting blood glucose levels at doses of 100mg and 10mg/kg body weight compared to untreated diabetic rats.
3. *X. americana* administered at 10mg, 100mg and 1000mg/kg body weight reduced fasting blood glucose in diabetic rats to levels that were not significantly different compared to the Glibenclamide treated rats. At 10mg and 100mg/kg body weight, fasting blood glucose levels were significantly different compared to untreated diabetic rats.
4. *Z. chalybeum*, *F. sycomorus* and *X. americana* stem bark extracts did not have any hypoglycemic effects in normal rats.
5. *Z. chalybeum*, *F. sycomorus* and *X. americana* stem bark extracts contain secondary metabolites that may be responsible for the antidiabetic effects observed.
6. Treatment of diabetic rats with *Z. chalybeum* and *F. sycomorus* significantly reduced serum alkaline phosphatase levels in a dose dependent manner, suggesting hepatoprotective effects.

7. No significant differences were observed in the hepatic and renal function indices between extract treated rats and normal controls thus the extract could be considered safe at the doses used.

5.3 RECOMMENDATIONS

Based on the results obtained, the following recommendations are proposed:

1. The efficacy of combined doses of the extracts should be determined to justify multiple plant therapy in the treatment of diabetes mellitus.
2. Further research is required to explore antidiabetic activity of the extracts at different dosages within the dosages used so as to determine the most efficacious dose for each extract.
3. Quantification of the phytochemical compounds associated with antihyperglycemic activity should be carried out to account for the differences in activity between the plant species.
4. The antidiabetic activity of the organic extracts of the plants should be investigated to determine the most efficacious solvent.
5. Long term toxicity of the plants should be investigated to determine the effects associated with long term use.
6. Conservation measures should be put in place and the traditional health practitioners educated on sustainable use as well as other possible sources for example cultivation of the medicinal plants.

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APPENDIX 1

EVALUATION OF MEDICINAL PLANT-PREPARATIONS USED IN THE TREATMENT OF TYPE II DIABETES MELLITUS

Serial number of the questionnaire……

Name of interviewer …… Date ……

PART ONE: CONSENT

A. RESEARCHER'S DECLARATION

1. The following research will be undertaken with respect to the indigenous knowledge and intellectual proprietary of the herbal practitioners.

2. We will at no given time initiate or conduct practices that are deemed to obtain information from the respondents by intimidation, coercion or false pretence.

3. We will be under no obligation to edit or tamper the information provided by the respondents.

4. The information collected will be used for the described research purpose only and not any undisclosed intentions.

Signatory Researchers:

1) Clare Njoki Kimani …….…….. Date ……

2) Dr. James Mbaria ……..…… Date …….

B: RESPONDENTS CONSENT AGREEMENT

I..... hereby agree to participate in this study with my full consent and conscience and declare that to the best of my knowledge the information that I have provided is true, accurate and complete.

Signature/Thumb print..... Date.....

PART TWO

A: BIODATA

Name…….……Age (yrs)……Gender...…...

Location of practice…….in …….……...Division

Number of years of practice …….

How did you acquire your skills?

……..

Level of education (None; Primary; Secondary; College; Other ……...

Contact(s)……

B: INFOFRMATION ON TRADITIONAL HERBAL PRACTICE

1. Do you treat diabetes mellitus? .....
2. Which types? .....

   .....
3. For the above condition (……..), what are the signs/symptoms or patient complains?

   .....

   .....

   .....

   .....
4. Number of cases treated in the last six months
5. When did you treat the last case (Last week/month/year)

6. Was the patient referred to you? By who?

7. Are any of the above conditions inherited?

8. Which gender is mostly affected? (1)Male (2) Female

C: MEDICINAL PLANTS USED

1. Which plants do you use to treat the above condition?

Vernacular name	**Ingredient (**whole plant, leaves, roots, seeds, flowers)	**Preparation/Quantity Used and method of administration**	**Level of confidence**
a.			
b.			
c.			
d.			
e.			
f.			
g.			

h.			
i.			
j.			

Key for confidence level: 1. High, 2. Medium, 3. Low

2. Which three plants do you most commonly use?

a)……...

b)……...

c)……

3. Which three plants are most effective? (In order starting with the most effective)

a)……...

b)……...

c)……

4. What side effects are associated with the plants in 3 above? (What happens if one takes too much of the medicine?)

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5. What do you give in case of the situation in question 5 above?

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6. What is the shelf life of the preparations in question 3 above?

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7. Where do you get your plants from?(cultivation/forests/other)

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8. How long does it take you to get to the source of the plants?

.....

9. Are the plants in large supply?

.....

.....

10. How do you know that treatment is effective?

a. Full recovery ☐

b. Partial recovery ☐

c. Symptomatic recovery ☐

11. How much do you charge for a single complete course of treatment?

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12. How often does one take the medicine? For how long?

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13. Are there any complementary measures required for treatment to be effective? (e.g. diet modification)……

……

……

14. What is the time required to make a full recovery after the onset of treatment?

.....

.....

15. Does the patient have to be on the treatment their entire life? (Details)

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16. What problems do you encounter in herbal medicine practice?

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