ANTIDIABETIC ACTIVITY AND SAFETY OF *PIPER CAPENSE, BERBERIS HOLSTII, SONCHUS ASPER, VERNONIA LASIOPUS AND GALINSOGA PARVIFLORA* IN ALLOXAN-INDUCED DIABETIC ALBINO MICE

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DECLARATION

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

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DEDICATION

I dedicate this thesis to my husband Daniel Muraya and my daughter Nicole Wambui Muraya from whom I get inspiration.

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ABBREVIATIONS AND ACRONYMS

A1C	Hemoglobin A1c test
ANOVA	Analysis of variance
CHD	Coronary heart disease
CVD	Cardiovascular disease
DKA	Diabetic ketoacidosis
DM	Diabetes Mellitus
DN	Diabetic nephropathy
DPP	Diabetes Prevention Program
DR	Diabetic retinopathy
ECM	Extracellular matrix
ESRD	End-stage renal disease
FPG	Fasting plasma glucose
GTT	Glucose tolerance test
Hb Alc	Glycosylated hemoglobin
HNC	Hyperosmolar non-ketotic coma
IDDM	Insulin dependent diabetes mellitus
IFG	Impaired fasting glucose
IGT	Impaired glucose tolerance
LA	Lactic acidosis
LADA	Latent autoimmune diabetes in adults
MODS	Maturity onset diabetes
NIDDM	non-insulin dependent diabetes mellitus

OGTT	Oral glucose tolerance test
PAD	Peripheral arterial disease
WHO	World Health Organization

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ABSTRACT

In Kenya, diabetes mellitus is of health concern to the public, because it causes substantial morbidity, mortality, and long-term complications. Synthetic drugs used in the management of diabetes are unavailable, have numerous side effects and are expensive. Many plants such as *Piper capense, Berberis holstii, Sonchus asper, Vernonia lasiopus* and *Galinsoga parviflora* used traditionally to manage many diseases including diabetes mellitus but their efficacy and safety after long-term use are not scientifically validated. This study aimed to determine *in vivo* antidiabetic activity and safety of aqueous plant extracts of *Piper capense, Berberis holstii, Sonchus asper, Vernonia lasiopus* and *Galinsoga parviflora* in male albino mice. Aqueous plant extracts were screened for antidiabetic activity in diabetic mice using the intraperitoneal and the oral routes. In the study, albino mice were assigned into eight groups of five mice each. For this purpose, reduction in blood glucose relative to their initial values was determined after oral and intraperitoneal administration of 25, 48.4, 93.5, 180.9, and 350 mg of aqueous extracts/kg body weight. 1IU/kg body weight dose of insulin and 4.6 mg of glibenclamide (200 mg/kg body weight) were used as a standard hypoglycemic agent to compare the results. Glucose levels were estimated at the beginning of the experiment and repeated after 2, 4, 6, 8, 10 and 24 hours after administering the drugs. Significant reduction in blood glucose relative to their initial values was determined for all treated non-diabetic and diabetic groups at the end of experiment. Mineral composition of the aqueous plant extracts was determined using TRXF (total reflection X-ray fluorescence system) while the quantities and types of phytochemicals present were determined using standard procedures. Toxicity of the aqueous plant extracts to normal mice was studied by orally and intraperitoneally administering them with 450, 670 and 1000 mg/kg body weight daily for 28 days and kept under close observation. Changes in body and organ weight, hematological and biochemical parameters were also determined. After the 28^{th} day, mice were sacrificed and pieces of pancreas, lungs, brain, testis, heart, kidney, spleen and livers were removed for weight change evaluation. Aqueous extracts orally and intraperitoneally administered at 25, 48.4, 93.5, 180.9, and 350 mg/kg body weight showed antidiabetic activity through either route. Oral and intraperitoneal dose of 450, 670 and 1000 mg/kg body weight of the plant extracts significantly reduced the body weight gain. The same oral and intraperitoneal dose of *Piper capense*, *Berberis holstii*, *Sonchus asper*, *Vernonia lasiopus* and *Galinsoga parviflora* altered the hemoglobin levels, mean cell hemoglobin concentration, platelets, red blood cell count, packed cell volume, mean cell volume, white blood cell count and their differential counts. The dose also altered activities of aspartate and alanine aminotransferases, alkaline phosphatase, α-amylase and lactate dehydrogenase. The plants extract contained phenols, tannins, saponins, flavonoids, and alkaloids. Minerals present were potassium, calcium, titanium, bromine, iron, zinc, copper, chromium, manganese, vanadium, rhubidium, strontium, and heavy metal lead. The observed antidiabetic activity toxicity observed in the plants extracts could be due to the phytochemicals and minerals present in the plants extracts. The study recommends use of safe plants with antidiabetic activity as herbal remedies. Comprehensive safety studied on the plants and organic solvent extraction for comparison of the activities of both organic and aqueous extracts.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Diabetes mellitus (DM) is a group of disorders that disturbs metabolism of fats, proteins and carbohydrates. It results from insufficient secretion of insulin or reduced sensitivity of tissues to insulin (Sides *et al.,* 2010). Characteristics of diabetes mellitus are elevated plasma glucose and ketoacidosis. If this disease is left uncontrolled, it can be fatal due to ketoacidosis. There are three main types of diabetes mellitus; diabetes type 1, diabetes type 2 and gestational diabetes (Sayed *et al.,* 2011).

Diabetes mellitus affects the citizens of both developed and developing countries. The disease affects an estimated 25% of the world population (Kavishankar et al., 2011). Prevalence of diabetes for all age groups worldwide is on the increase and will be the world's main killer and disabler in the next 25 years (Edwin *et al.,* 2006). Diabetes mellitus is becoming a serious threat to mankind. Estimated prevalence of diabetes in Kenya ranges between 10.7% (urban) and 2.7% (rural). Diabetes accounted for 27% of hospital admissions in 2003 (Kavishankar *et al.,* 2011).

Diabetes has been conventionally managed with conventional drugs that function as hypoglycemic agents. Oral hypoglycemic agents especially biguanides and sulphonylureas have been employed in the treatment and management of especially type 2 diabetes. These drugs are expensive and pose serious negative effects to the potential users. These reasons, therefore, neccesitate search for safe and cheap blood

glucose lowering oral hypoglycemic agents that would be effective in the treatment of diabetes and without serious side effects. Herbs have been seen as the best option (Ogbonnia *et al.,* 2008). The use of plant-derived compounds as part of herbal preparations for alternative sources of medicament continues to play major roles in chemotherapy (Joy *et al.,* 1998). Several studies have shown that traditional medicines could provide better glycemic control than the currently used conventional drugs. In many regions of the world, herbal remedies continue to be more accessible and affordable than conventional drugs (Okpara *et al.,* 2007).

Most herbal medicines used in the developing countries have not been evaluated for efficiency, safety and quality. Efficacy and safety of herbs therefore need to be assessed (Hayelom *et al.,* 2012). Testing herbs in animal models gives important information on the type of extract to be made, a suitable dose, the likely toxic effects and the efficacy. The information is then translated to human studies (Amala, 2006).

1.2 Statement of the Research Problem and Justification of the study

The world survey indicates that diabetes mellitus is affecting nearly 25% of the population (Kavishankar *et al.,* 2011). The prevalence of diabetes worldwide is on the increase and is projected to be the World's main killer in the next 25 years (Edwin *et al.,* 2006). The estimated diabetes prevalence in Kenya ranges between 10.7% (urban) and 2.7% (rural). In 2003, diabetes accounted for 27% of hospital admissions (KDMIC, 2012). DM is the main cause of hospitalization and disability hence resulting in significant financial burden to the families (Vats *et al.,* 2002).

Synthetic hypoglycemic drugs have been used in the treatment of DM. However, there are limitations in the currently available anti-diabetic drugs in terms of efficacy, safety and high cost (Holman and Turner, 1991). It is now evident that most of the currently used synthetic drugs for the treatment of diabetes mellitus are not affordable to low income earners and not easily accessed by the users. This is also coupled with their serious side effects posed to the potential users (Rang *et al.,* 1991).

Insulin used in the management DM has several limitations like fatty liver, brain atrophy, anorexia nervosa and insulin resistance.(Yaryura *et al.,* 2001). Chronic treatment with oral hypoglycemic agents such as biguanides and sulfonylurea are associated with negative effects (Rang *et al.,* 1991). Search for appropriate antidiabetic agents has been narrowed on plants used as traditional drugs due to leads provided by natural products that may be better treatment. Increasing prevalence of the disease in rural population is a clear indicator of the need for development of inexpensive herbal sources of anti-diabetic drugs (Ven and Pari, 2003). Herbal drugs have fewer side effects and less toxic compared to synthetic drugs, hence there is interest all over to explore alternative therapies which are believed to be safer and economical (Ernst, 1998). This, therefore, led to the need for research into alternative medicine that retain therapeutic efficacy and can be taken for long durations without or with minimal side effects and with low prescription cost. Amongst many herbs used for treatment of DM, few have been validated by scientific criteria (Frode and Medeiros, 2008). An investigation of herbs for diabetes may provide important information for the development alternative drugs. In this context it was found that;

Piper capense, Berberis holstii, Sonchus asper, Vernonia lasiopus, Galinsoga parviflora, have been used in treatment of diabetes, but their use has not been validated scientifically. The study therefore is aimed at evaluating them in terms of efficacy and toxicity.

1.3 Research Hypotheses

1. The aqueous herbal extracts of *Sonchus asper, Vernonia lasiopus, Galinsoga parviflora, Piper capense* and *Berberis holstii* have no antidiabetic activity in alloxan induced diabetic mice.
2. The aqueous herbal extracts of *Sonchus asper, Vernonia lasiopus, Galinsoga parviflora, Piper capense* and *Berberis holstii* have no toxic effects on normal mice.

1.4.1 General objective

To evaluate hypoglycemic activities and safety of aqueous extracts of *Sonchus asper, Vernonia lasiopus, Galinsoga parviflora, Piper capense* and *Berberis holstii.*

1.4.2 Specific objectives

1. To determine the effects of aqueous extracts of *Sonchus asper, Vernonia lasiopus, Galinsoga parviflora, Piper capense* and *Berberis holstii* on blood glucose levels in alloxan-induced diabetic mice.

2. To determine the phytochemical and mineral composition of the aqueous extracts of *Sonchus asper, Vernonia lasiopus, Galinsoga parviflora, Piper capense* and *Berberis holstii*.
3. To determine the most effective route of administration of herbal extracts of *Sonchus asper*, *Vernonia lasiopus, Galinsoga parviflora, Piper capense* and *Berberis holstii* in diabetic mice.
4. To determine any toxic effects of high doses of the aqueous roots and leaves extracts of *Sonchus asper, Vernonia lasiopus, Galinsoga parviflora, Piper capense* and *Berberis holstii* on normal mice.

CHAPTER TWO

LITERATURE REVIEW

2.1 Diabetes

Diabetes is a condition marked by abnormally high levels of glucose in the blood. People with diabetes either produce insufficient insulin or their tissue cannot be able to use the insulin that their bodies produce. As a result, sugar builds up in their blood (Piero *et al.*, 2015). Increased blood sugar leads to polyuria polydipsia and polyphagia. The main types of DM are type 1, type 2 and gestational diabetes.

2.2 Prevalence and Incidence of Diabetes Mellitus

Currently, the overall prevalence of DM is between 3.0% and 3.6% of the world population. Type 2 constitutes 90% these worlds population. Prevalence of DM for all age groups was estimated to be 2.0% in 1997 (Amos *et al.*, 1997) and was projected to be 3.6% in 2010 and 4.4% in 2030 (Wild *et al.,* 2004). The diabetes prevalence is reported to be higher in men than in women. Urbanization, population growth, increasing physical inactivity and prevalence of obesity factors contribute to the increasing prevalence of type 2 diabetes mellitus (Wild *et al.,* 2004).

2.3 Signs and Symptoms

Persons with type I diabetes experience the following symptoms; polyuria, and polydipsia (extreme thirst). This is due to increased blood glucose, which causes kidneys to create urine above normal amount. Losing fluid makes a person dehydrated. Dehydration leads to thirsty (Rhomesh, 1994). Weight loss, without loss

of appetite, occurs due to dehydration (Romesh, 1994). Other symptoms are, extreme hunger, weakness, nausea, numbness of arms, tiredness, dry skin, vomiting, abdominal pain, more infections than usual. Dehydration causes fatigue, weakness and confusion (Romesh, 1994).

Type II diabetes often have no symptoms, and the condition is detected when a routine examination show high sugar level in the blood. Half of the population with type II diabetes does not show symptoms. The disease is diagnosed years after its onset; when complications are already present (DRWF, 2011). People with type II diabetes may experience symptoms such as, numbness of limbs, poor vision; impotence; poor wound healing and Fatigue (Donner *et al*., 2009). Half of people with type II diabetes don't show any symptoms and hence don't know that they have diabetes (DRWF, 2011).

2.4 Types of Diabetes Mellitus

2.4.1 Type I Diabetes Mellitus

Type I DM results from the failure of the body to produce insulin. This form is also referred to as "insulin-dependent diabetes mellitus" (IDDM). Type I DM result from illness or stress that causes the immune system to attack and destroy the insulin producing cells of the pancreas. This results in the pancrease not producing insulin. The management for type 1 diabetes is to take insulin injection every day. Type 1 develops suddenly in childhood or Adolescence (Shukla *et al*., 2011).

2.4.2 Type II Diabetes Mellitus

Type 2 diabetes mellitus results from resistance to insulin. This form is also referred to as non-insulin dependent diabetes mellitus (NIDDM). Type II diabetes is more common than type 1 and approximately 90% of all diabetes cases are type 2 (Shukla *et al.*, 2011).

2.4.3 Gestational Diabetes

Gestational diabetes develops only during pregnancy and affects 2 to 10 percent of all pregnancies. It can complicate pregnancy leading to prenatal morbidity and mortality, so clinical detection is important (NDIC, 2012). Gestational diabetes is fully treatable but requires careful medical supervision throughout the pregnancy.

2.4.4 Other Specific Types of Diabetes

Other types of diabetes includes steroid diabetes, congenital diabetes, secretion, cystic fibrosis-related diabetes, and several forms of monogenic diabetes (Shukla *et al.*, 2011).

2.4.5 Pre-diabetes

Pre-diabetes occurs in individuals with blood sugar levels that are above normal but not diabeteic level. This raises the risk of developing type II diabetes. Pre-diabetes is also called impaired fasting glucose (IFG), impaired glucose tolerance (IGT), or insulin resistance (Batty *et al.*, 2007).

2.5 Complications of Diabetes Mellitus

Pathological features are due to the following factors (Shukla *et al.,* 2011): Decrease in use of glucose by the body cells which leads to increase in blood sugar concentration; Increase in movement of fats from the fat storage areas which results in abnormal fat metabolism and deposition of cholesterol in arterial walls causing atherosclerosis (Amreen *et al.,* 2012).

2.5.1 Acute Complications of Diabetes Mellitus

The acute complications of DM includes; hyperomolar non-ketotic coma (HNC), lactic acidosis (LA), hypoglycemia and diabetic ketoacidosis (DKA), hyperosmolar non-ketotic coma (HNC), lactic acidosis (LA) and hypoglycemia (Fishbein, 1995).

2.5.1.1 Diabetic ketoacidosis (DKA)

Diabetic ketoacidosis results from deficiency of insulin an effect of increased levels of counter-regulatory hormones glucagon, cortisol, growth hormone and catecholamines (Kitachi *et al.,* 2001). Diabetic ketoacidosis has characteritic such as severe depletion of electrolytes and water (Wolfsdorf *et al.,* 2007). Diagnostic parameter of DKA includes, hyperglycemia > 250mg/dl; serum bicarbonate < 15mmol/L or venous pH < 7.3; or serum and ketonuria and ketonemia (Dunger *et al.,* 2004).

2 .5.1.2 Hyperosmolar non-ketotic coma

Hyperosmolar non-ketotic coma is clinically distinct by insulin deficiency and hyperglycemia usually > 1,000 mg/dl with associated increased serum osmolality, dehydration and stupor progressing to coma if uncorrected (Arieff and Carroll, 1972).

2.5.1.3 Lactic acidosis (LA)

LA comprises lactic acidemia ≥ 2.0 mmol/L with acidosis (pH ≤ 7.3) and without ketoacids. Approximately half of the cases of lactic acidosis are present in patients with diabetes (Kreisberg, 1980).

2.5.1.4 Hypoglycaemia

Hypoglycemic reactions are most common complication of insulin therapy include Tachycardia, palpitation, sweating, nausea and hunger are common sign of hypoglycaemia (Katzungs *et al.,* 2009).

2.5.2 Chronic Complications of Diabetes Mellitus

Injurious effects of increased blood sugar are separated into microvascular and macrovascular complications.

2.5.2.1 Microvascular complications of diabetes

Microvascular complications are: Vascular changes in diabetes, diabetic retinopathy, diabetic nephropathy and diabetic neuropathy.

i) **Vascular changes in diabetes**

Changes in the vasculature occur before the onset of overt diabetes. Basement membrane thickening is common in retinopathy, nephropathy and neuropathy, which may be a direct consequence of the expression and deposition of extracellular matrix (ECM) proteins in the vasculature (Williamson and Kilo, 1983).

ii) **Diabetic retinopathy**

This is characterized by features such as thickening of pericyte, vascular basement membrane, vascular microaneurysms, pathologic neovascularization and vascular occlusion that laeds to retinal hemorrhage, retinal detachment and vision loss (Yaron *et al.,* 1992). Severity and duration of hyperglycemia are linked directly to pathologic changes and cause vascular dysfunction, such as, retinal angiogenesis and vascular permeability (Robison *et al.,* 1991). Diabetic retinopathy (DR) in form of macular edema has been summarized as the common cause of blindness, which is the most feared complication of diabetes. It is preventable, mostly in the age group of 30-74 years. DR is due to microangiopathy affecting the retinal pre-capillary arterioles, capillaries and venules. Detection of earliest signs is essential for treatment strategies (Grassi, 2003).

iii) **Diabetic nephropathy**

Diabetic nephropathy is characterized by microalbuminuria, which turns to albuminuria indicating severe renal dysfunction and renal failure (Drummond and Mauer, 2002) and is the leading cause of, ESRD (end-stage renal disease) (Brenner *et*

al., 2001) necessitating long term dialysis or the kidney transplantation (Gordois *et al.*, 2004). Diabetic nephropathy has become the main cause of mortality among the young adults with diabetes and in adult's persistent micro-albuminuria is the best marker of consequent risk for its development (Schultz *et al.*, 1999).

iv) **Diabetic neuropathy**

Neuropathy associated with diabetes is characterized by the presence of signs and symptoms of peripheral nerve dysfunction in diabetic people (Boulton *et al*., 1998). The three proposed stages of neuropathy include the functional stage which is reversible, alteration of nerve functioning; structural phase which may be reversible, loss of structural changes in fibers of the nerves and finally death of the nerve that is characterized by irreversible decrease in the density of nerve fiber and death of neuron death (Vink, 2002). Risk factors for diabetic neuropathy are; cardiovascular disease (CVD), microalbuminuria and severe ketoacidosis (Tesfaye, 1996). Diabetic foot is due to diabetic neuropathy, macro-angiopathy or a combination of both. Triggering factors of diabetic neuropathy are foreign bodies, exogenous trauma by tight shoes, and insufficient care, together with deformity of foot. Pathogenic factors included decreased collagen deposition, loss of adipose tissues and occurrence of edema (Stiegler, 2004).

2.5.2.2 Macrovascular complications of diabetes

The diabetic macrovascular disease is increased by atherosclerosis resulting in risks of myocardial infarction, lower extremity gangrene and stroke (Maitra and Abbas, 2005).

These complications include cardiovascular complication of diabetes, peripheral arterial disease and cerebrovascular complication of diabetes.

i**) Cardiovascular Complications of diabetes**

Patients with diabetes have high chances of developing CDV (cardiovascular disease) than those without (ADA, 2005). Risk factors that may contribute to the development of CHD (coronary heart disease) include sedetary lifestyle, hypertension, cholesterol and hyperglycemia. Biochemical mechanisms leading to worse outcomes of CVD include hypercoagulability, endothelial dysfunction, fibrinolysis, impaired platelet, oxidative stress, hyperaggregability, glucose toxicity and sympathovagal imbalance (Hafner, 2005).

ii) **Peripheral arterial disease (PAD)**

PAD is an occlusive disease of atheroseclerosis. It is risk factor for lower limbs amputations. The abnormal metabolic due to diabetes leads to changes in the arterial structure, state and function predisposing individuals to peripheral arterial disease (Creager and Libby, 2001). The risk of peripheral arterial disease development increases in patients with diabetes (Murabito *et al.,* 1997).

iii) **Cerebrovascular complications of diabetes**

Different terms are used to describe cerebrovascular events. These are transient ischemic attack, which is a condition in which a patient experiences a temporary focal

neurologic deficit. Warning signs of cerebrovascular events are loss of coordination, unilateral weakness, sudden confusion, and numbness (Welty, 2011).

2.6 Causes Diabetes

2.6.1 Causes of Type I Diabetes

Type I diabetes is coused by environmental factors, infant Feeding Practices, and autoimmune destruction of beta cells.

2.6.1.1 Autoimmune destruction of beta cells

White blood cells called T cells attack and destroy beta cells in type 1 diabetes. This process begins before diabetes symptoms appear and continues after diagnosis. The immune systems of people who are susceptible to developing type I diabetes respond to insulin as if it were a foreign substance, or antigen (NDIC, 2011). To combat antigens, the body makes antibodies. Antibodies to insulin and other proteins produced by beta cells are found in people with type I diabetes (Alan, 2011).

2.6.1.2 Environmental factors

Environmental factors, such as foods, viruses, and toxins, play a role in the development of type I diabetes, but the exact nature of their role has not been determined. Some theories suggest that environmental factors trigger the autoimmune destruction of beta cells in people with a genetic susceptibility to diabetes. Other theories suggest that environmental factors play an ongoing role in diabetes, even after diagnosis (Alan, 2011).

2.6.1.3 Infant Feeding Practices

Some studies have suggested that dietary factors may raise or lower the risk of developing type1 diabetes. For example, breastfed infants and infants receiving vitamin D supplements may have a reduced risk of developing type I diabetes, while early exposure to cow's milk and cereal proteins may increase the risk. More research is needed to clarify how infant nutrition affects the risk for type I diabetes (NDIC, 2011).

2.6.2 Causes of Diabetes Type II

2.6.2.1 Genetic susceptibility

Genes play a significant part in susceptibility to type II diabetes. Having certain genes or combinations of genes may increase or decrease a person's risk for developing the disease. Recent studies have combined genetic data from large numbers of people, accelerating the pace of gene discovery. Though scientists have now identified many gene variants that increase susceptibility to type II diabetes, the majority have yet to be discovered. The known genes appear to affect insulin production rather than insulin resistance. Researchers are working to identify additional gene variants and to learn how they interact with one another and with environmental factors to cause diabetes (NDIC, 2011).

2.6.2.2 Obesity and physical inactivity

Physical inactivity and obesity are strongly associated with the development of type II diabetes. People who are genetically susceptible to type II diabetes are more

vulnerable when these risk factors are present. An imbalance between caloric intake and physical activity can lead to obesity, which causes insulin resistance and is common in people with type II diabetes. Central obesity, in which a person has excess abdominal fat, is a major risk factor not only for insulin resistance and type II diabetes but also for heart and blood vessel disease, also called cardiovascular disease (CVD). This excess "belly fat" produces hormones and other substances that can cause harmful, chronic effects in the body such as damage to blood vessels. The DPP and other studies show that millions of people can lower their risk for type II diabetes by making lifestyle changes and losing weight (NDIC, 2011).

2.6.2.3 Insulin resistance

Insulin resistance is a common condition in people who are overweight or obese, have excess abdominal fat, and are not physically active. Muscle, fat, and liver cells stop responding properly to insulin, forcing the pancreas to compensate by producing extra insulin. As long as beta cells are able to produce enough insulin, blood glucose levels stay in the normal range. But when insulin production falters because of beta cell dysfunction, glucose levels rise, leading to prediabetes or diabetes (NDIC, 2011).

2.6.2.4 Abnormal glucose production by the liver

In some people with diabetes, an abnormal increase in glucose production by the liver also contributes to high blood glucose levels. Normally, the pancreas releases the hormone glucagon when blood glucose and insulin levels are low. Glucagon stimulates the liver to produce glucose and release it into the bloodstream. However,

when blood glucose and insulin levels are high after a meal, glucagon levels drop, and the liver stores excess glucose for later. For reasons not completely understood, in many people with diabetes, glucagon levels stay higher than needed. High glucagon levels cause the liver to produce unneeded glucose, which contributes to high blood glucose levels (NDIC, 2011).

2.7 Diagnosis of Diabetes

Early screening of diabetes allow for the identification of at-risk persons and those with early disease. There are three tests that can diagnose diabetes: Oral glucose tolerance test (OGTT), hemoglobin A1C (A1C) and Fasting plasma glucose (FPG).

2.7.1 Fasting plasma glucose test

The fasting plasma glucose (FPG) is a simple blood test taken after 8 hours of fasting. FPG levels indicate: Normal (5.5 mmol/L), pre-diabetes or a risk factor for type 2 diabetes (5.5 - 7.0 mmol/L) and diabetes (7.0 mmol/L or higher) (Harvey, 2012).

2.7.2 Oral glucose tolerance test (OGTT)

OGTT is used in diagnosing prediabetes, diabetes, and gestational diabetes. OGTT has been shown to be more sensitive than the FPG test, but it is less convenient to administer. OGTT measures blood glucose after a person fasts for at least 8 hours and 2 hours after the person drinks a liquid containing 75 g of glucose in water. If the 2-hour blood glucose level is between 140 and 199 mg/dL, the person has a type of prediabetes called impaired glucose tolerance (IGT). If confirmed by a second test, a

2-hour glucose level of 200 mg/dL or above means a person has diabetes (David, 2012).

2.7.3 A1C test

The A1C test is only recommeded to detect type II diabetes and prediabetes. A1C test reflects the average person's blood glucose levels over the past 3 months but does not show daily fluctuations. The A1C test is the most convenient test for the patients than the traditional glucose tests because no fasting is required. A normal A1C level is below 5.7 percent. An A1C of 5.7 to 6.4 percent indicates prediabetes. People diagnosed with prediabetes are retested in 1 year. People with an A1C above 6.0 percent are at very high risk of developing diabetes. A level of 6.5 percent or above means a person has diabetes (David, 2012).

2.7.4 Autoantibody tests

The presence of a variety of antibodies that attack beta cells of pancrease characterizes type I diabetes. These antibodies are known as autoantibodies because they attack the body's own cells. Blood tests for these autoantibodies can help differentiate between type I and type II diabetes (Hervey, 2012).

2.8 The Roles of Insulin and Glucagon in Normal Blood Glucose Regulation

A healthy person's body keeps normal range of blood glucose levels through several mechanisms. The two hormones insulin and glucagon, made in the pancreas, helps to regulate blood glucose levels. Insulin, made by beta cells lowers elevated blood

glucose levels while glucagon, made by alpha cells, raises low blood glucose levels. Insulin helps muscle, fat, and liver cells absorb glucose from the bloodstream, lowering blood glucose levels. Insulin stimulates the liver and muscle tissue to store excess glucose in form of glycogen (Amreem, 2012). Insulin also lowers blood glucose levels by reducing glucose production in the liver. When blood glucose levels drop, the pancreas releases glucagon into the blood. Glucagon signals the liver and muscle tissue to break down glycogen into glucose, which enters the bloodstream and raises blood glucose levels. If the body needs more glucose, glucagon stimulates the liver to make glucose from amino acids (Amreem, 2012).

2.9 The Mechanism of Alloxan Action on β-cells of Mice Pancreas

Alloxan is used to induce experimental diabetes in animals. The cytotoxic action of these diabetogenic agents is mediated by reactive oxygen species (ROS). Alloxan and the product of its reduction, dialuric acid, establish a redox cycle with the formation of superoxide radicals. These radicals undergo dismutation to hydrogen peroxide. Thereafter highly reactive hydroxyl radicals are formed by the Fenton reaction. The action of reactive oxygen species with a simultaneous massive increase in cytosolic calcium concentration causes rapid destruction of β-cells (Szkudelski, 2001).

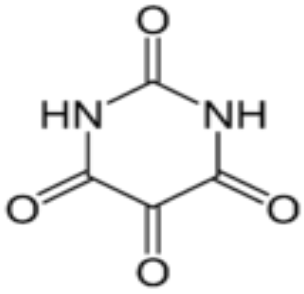

Figure 2.1 The chemical structure of alloxan

2.10 Management of Diabetes

Management of diabetes is done to reduce the symptoms and reduce blood sugar levels as uncontrolled high blood sugar level is associated with risks of diabetic complications. Type I diabetes require treatment with insulin while type II diabetes, treatment is diet modification and lifestyle change with oral hypoglycemic agents are required as the disease progresses (Holden and Currie, 2012). A number of therapeutic choices are available for the management of the disease; however, none are free of disadvantages.

2.10.1 Insulin Therapy

The overall effect of diabetes treatment with insulin (injectables and secretagogues) is increased insulin response to glycemic changes and hepatic glucose absorption (Frier and Fisher, 2010). Previously, insulin was obtained by extraction and purification from the pancreas of pigs and cows (porcine and bovine insulin respectively) and some patients still prefer animal insulin. Large-scale production of human insulin has been enabled by recombinant DNA technology. Recently, insulin's sequence of amino has been altered to produce insulin analogues, which differ in their rate of absorption from the site of injection (Frier and Fisher, 2010).

High glucose concentrations in blood triggers secretion of insulin. Other factors that influence insulin secretion in the body include gene expression, hormonal regulation as well as cAMP levels. The primary function of insulin in the body is the reduction of

glucose levels in blood. It is however also involved in protein, lipid, carbohydrate as well as glucagon metabolic processes (Nelson and Cox, 2014).

Insulin exerts its action via its receptor. Insulin has two identical alpha chains and two transmembrane units as well as disulphide bonds. Insulin binds to the alpha sub units on the insulin receptor, stimulating tyrosine kinase enzyme. This enzyme initiates the phosphorylation mechanism that leads to the translocation on of glucose transporter 4 (GLUT 4), which in turn increases glucose uptake (Greenspan *et al.,* 1997). The insulin receptor is also responsible for mediating post receptor signaling pathways whose function is to further regulate glucose homeostasis in the body. This is done via the phosphatidyl inositol-3-kinase (PI-3-K) activity as well as the mitogen activating protein kinase (MAPK) pathway (Schenk *et al.,* 2008).

Insulin injection is done subcutaneously several times daily into the upper arms, abdominal wall, buttocks and outer thighs (Frier and Fisher, 2010). Inhaled insulin introduced in 2006 has an onset of action similar to rapid-acting insulin analogs with duration of glucose-lowering activity comparable to subcutaneously administered regular human insulin (Frier and Fisher, 2010).

Patients with type I diabetes require lifelong treatment with insulin to promote glucose utilization. Optimal diabetic control requires frequent self-monitoring of blood glucose. Frequent monitoring allows for rational adjustments in insulin doses. A multiple injection regimen, in which regular or lispro insulin is adjusted before each meal and intermediate-acting insulin given at bedtime, is used to provide more

flexibility and achieve better glycemic control. With a multiple injection regimen, patients add or subtract regular or lispro insulin (called compensatory doses) from their basic insulin dose in response to the immediate blood glucose level before the meal (Piero *et al.*, 2012d).

The complications of insulin therapy include: hypoglycemia, weight gain, peripheral edema (insulin treatment causes salt and water retention in the short term), insulin antibodies (animal insulins), local allergy (rare) and lipodystrophy at injection sites (Frier and Fisher, 2010).

2.10.2 Oral Hypoglycemic Drugs

There are five classes of oral agents approved for the treatment of diabetes. These are biguanide, thiazolidinedione, sulfonylurea, α-glucosidase inhibitor, and meglitinide (Pandey *et al*., 2011).

2.10.2.1 Sulphonylureas

All sulphonylureas drugs have a sulphonic acid-urea nucleus, and different chemical moieties are added at various positions on the nucleus to make different drugs (Malender, 2004).

Sulphonylureas reduce blood sugar by increasing secretion of insulin from pancreatic β-cells in patients with residual β-cell function. They are well absorbed and their half-life and duration of action vary by agent (Piero *et al*, 2012d). Sulphonylureas bind to

sulphonylurea receptors on the surface of pancreatic β-cells, and cause the voltage-dependent potassium adenosine triphosphate channels to close, which facilitates cell-membrane depolarization, calcium entry into the cell, and insulin secretion. Sulfonylurea therapy reduces HbA1c levels by 1% to 2% (AACE, 2007).

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

2.10.2.2 **Biguanides**

Metformin is the only biguanide available. Metformin can trace its roots back to medieval Europe, where biguanides in the form of French lilac were used in diabetes treatment. Metformin is usually the first-line medication used for treatment of type II diabetes. In general, it is prescribed at initial diagnosis in conjunction with exercise and weight loss, as opposed to in the past, where it was prescribed after diet and exercise had failed. Biguanides such as metformin act by increasing glucose transport

across cell membrane of the skeletal muscle. They act in the presence of endogenous insulin, and are effective only where there are residual functioning pancreatic islet cells (Zhou *et al*., 2009). It affects primarily fasting glycemia; however, some decreases in postprandial glucose concentrations, especially after the midday meal, can also be seen. Metformin originally derived from a medicinal plant *Galega officinalis* (Piero *et al.*, 2012d). It has been shown to lower HbA1c levels by 1% to 2% and also confers other non-glycemic benefits such as decreasing low-density lipoprotein cholesterol (LDL-C) levels, triglyceride levels (AACE, 2007). Metformin is well tolerated with the most common side effect being gastrointestinal (GI) complaints, such as diarrhea, nausea, abdominal discomfort, and a metallic taste. All of these improve with time and dose reduction (American Diabetes Association, 2009).

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

2.10.2.3 α-Glucosidase Inhibitor

Alpha glucosidase inhibitors act by inhibiting alpha glucosidase enzyme in the brush border of the small intestine. This delays the absorption of glucose by decreasing the breakdown of complex carbohydrates by enteric digestive enzymes (Shibao *et al.*, 2007). Undigested sugar is delivered to the colon, where it is converted into short-chain fatty acids, methane, carbon dioxide, and hydrogen (Piero *et al.*, 2012d).

Alpha glucosidase inhibitors are contraindicated in patients with inflammatory bowel disease, partial intestinal obstruction, a predisposition to intestinal obstruction, colonic ulceration and other gastrointestinal disorders. Dose-dependent hepatotoxicity is associated with this drug class; so liver function tests are carefully monitored in patients receiving higher dosages of these medications (for example, more than 50 mg three times daily). Serum transaminase levels are checked every three months for the first year patients take the medication and periodically thereafter. However, their major side effects are gas, bloating and diarrhea (Piero *et al.*, 2012d).

2.10.2.4 Thiazolidinedione

Thiazolidinediones are known to act by increasing the sensitivity of peripheral tissues to insulin by affecting the expression of specific genes. They achieve this by binding and activating γ peroxisome proliferator-activated receptor (PPAR-γ), a nuclear receptor (Qatanani and Lazar, 2007). When activated, this receptor binds to response elements on DNA and alters transcription of various genes that regulate carbohydrate and lipid metabolism. Through this process, thiazolidinediones increase insulin-

stimulated glucose uptake in skeletal muscle cells (AACE, 2007). The high affinity of this drug to PPAR-γ is important in the management of insulin resistance since large adipocytes that differentiate from smaller ones produce TNF-α which increase insulin resistance. Thiazolidinediones therefore suppresses the expression of these adipokines involved in insulin resistance (Sharma and Staels, 2007).

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

2.10.2.5 Incretin-Based Therapies

Incretin-based therapies can be used as injections (GLP-1 analogs) or as pills (DPP-4 inhibitors). In part, this causes by secretion of gut hormones, or incretins, which potentiate glucose induced insulin secretion. Glucagon-like peptide (GLP-1) is an incretin hormone, which stimulates insulin secretion in a glucose-dependent manner. In addition, GLP-1 suppresses glucagon secretion, delays gastric empting, reduces appetite and encourages weight loss. As GLP-1 is rapidly degraded by enzyme dipeptidyl peptidase 4, inhibitors of this enzyme prolong its biological effect. The DPP-4 inhibitors or gliptins (sitagliptin, vildagliptin, and saxagliptin) are oral agents which act in this manner (Frier and Fisher, 2010).

All incretin-based medications carry increased risk of acute pancreatitis. Patients must be warned about this risk and be advised to stop taking these medications and seek medical evaluation if acute abdominal pain develops. These medications should not be given to the individuals who have a history of medullary thyroid carcinomas or have multiple endocrine neoplasia syndrome type II. This is because increased incidences of the thyroid C-cell tumors have been observed with these medications in experimental mice and rats. So far, there is no increased risk in humans but the above groups of individuals should not use these medications. *In vitro* and *in vivo* animal models suggest that glucagon-like peptide 1 promotes proliferation and neogenesis from precursor β-cells; however, this has not yet been demonstrated in humans treated with glucagon-like peptide 1 or exenatide (Frier and Fisher, 2010).

2.11 Herbs and Diabetes Management

2.11.1 Significance

An ethno-botanical study aims to add value on the information that already exists about a variety of local plants known to have specific medicinal properties. Plants produce a wide range of secondary metabolites that are bioactive which provide them with protection against external aggression from bacteria, fungi, viruses and insect pests. This then suggests that plants contain biologically active substances that can be used to protect humans, livestock, and crop plants against microbial and other disease causing agents. The possibility of relying on plant extracts for use in medicine has been and is continually being investigated (Day, 1998).

Many plants are however still unexplored, with many unique and potentially useful medicinal properties. Although scientific data is still lacking for the bulk of these plants, there are records of medicinal plant uses in various herbariums as well as institutions of learning. Most of the knowledge is however held by local herbalists who pass it on from generation to generation. In this regard there have been concerted efforts by governments as well as communities to put together a database of the traditional plants in use in many countries (Day, 1998). Although traditional herbalists may not be familiar with the active biological compounds in the medicinal plants they use, knowledge of such bioactive compounds would enhance the application and effectiveness of traditional herbal medicines against a wide range of human and animal ailments (Day, 1998). Not all the chemical compounds that are produced by or constitute the plants are of interest medicinally (Evans *et al.*, 1992).

There are many plants and plants extracts which possess marked hypoglycemic activity. Biological actions of the plant products used as alternative medicines to treat diabetes are related to their chemical composition. Herbal products or plant products are rich in phenolic compounds, flavonoids, terpenoids, coumarins, and other constituents, which show reduction in blood glucose levels (Rao *et al.*, 2010). Anecdotal as well as ethno-botanical data appears to support the use of traditional medicines by traditional health practitioners for diabetes syndrome. Some have shown remarkable results in decreasing blood sugar levels as well as mediating other cellular metabolic functions that generally enhance the diabetic patients overall health status. It is therefore prudent to do extensive research on the various plants in use and test their

efficacy and document their phytochemical compositions and mode of action (Day, 1998).

The mode of action of the extracts from these plants is uncertain. However, many anti-diabetic plants act, at least in part, through their fiber, vitamin or mineral contents and some secondary metabolites (Day, 1998). Mineral deficiencies are common in diabetic patients and aggravate insulin deficiency. Several minerals found in some medicinal plants are cofactors that signal intermediaries of insulin action and key enzymes of glucose metabolism (Day, 1998). Interest in and the research for medicines from natural sources has served as catalyst for exploring techniques of obtaining the required plants and probing their activities. Various parts of the plants are used by traditional medicine practitioners in the management and treatment of several disorders including cancer, rheumatism, hypertension and inflammatory diseases among others (Atawodi, 2005).

A notable number of modern drugs have been synthesized from these natural medicinal plants. These drugs are based on the indigenous medicinal information of plants. This natural source has been used to cure various diseases throughout the world. Actually, plants have great diversity of bioactive compounds and it is an indication which makes plants a prosperous source of different types of drugs (Mahmood *et al.*, 2012). The following are some of the most common antidiabetic plants used in the tradition medicine:

2.11.2 Garlic (*Allium sativum*)

It consists of bulbs of the plant known as *Allium sativum*, belonging to the Liliaceae. Volatile oil present in garlic is the chief active constituent, and contains allyl propyl disulphide, diallyl disulphide, alliin and allicin. Garlic cloves lower blood sugar significantly. Garlic is effective in lowering serum glucose levels in STZ-induced as well as alloxan-induced diabetic rats and mice through increased insulin secretion and increased insulin sensitivity (Liu *et al*, 2006; Londhe *et al*, 2011).

2.11.3 Neem (*Azadirachta indica)*

Azadirachta indica, belong to the family Meliaceae. Aqueous leaves extracts of neem significantly decreases blood sugar level and prevents adrenaline as well as glucose-induced hyperglycemia (Pandey *et al.*, 2011). The aqueous leaf extract orally administered decreased blood glucose levels in STZ-induced diabetes rats. Aqueous fruit extracts of neem reduced the normal blood glucose levels in normoglycemic rabbits (Rasineni *et al.*, 2008).

2.11.4 Onion *(Allium cepa)*

Allium cepa, belongs to the family Liliaceae. APDS an active ingredient in onion blocks the breakdown of insulin by the liver and stimulates insulin production by the pancreas, thus increasing the amount of insulin and reducing sugar levels in the blood. So, liberal use of onion is recommended for diabetes patients. The antidiabetic effect of onion has been reported by several studies using experimental animal models as well as human diabetes patients (Kook *et al.*, 2009; Ogunmodede *et al.*, 2012).

2.11.5 *Salacia oblonga*

Salacia oblonga, belongs to the family Hippocrateaceae and used as anti-diabetic herb. It binds to intestinal enzymes α-glucosidases that break down carbohydrate into glucose in body. Scientific work has shown S*alacia oblonga* have antidiabetic potential (Pallab and Amartya, 2012).

2.11.6 *Aloe vera*

Aloe vera has long been used all over the world for its various medicinal properties. Medicinal products are made from the mucilaginous tissue known as *Aloe vera* gel. The peripheral bundle of sheath cells produce intensely bitter, yellow latex, commonly termed aloe juice, or sap or aloes (Yagi *et al*, 2009). The antidiabetic effect of *A.vera* have been confirmed in experimental animals (Gupta *et al.,* 2011; Kavishankar *et al.*, 2011).

2.11.7 Dandelion (*Taraxacum officinale*)

T. officinale belongs to the family Asteraceae and is rich in triterpenoids, sterols, tannins, alkaloids, flavonoids (apigenin). Tests on diabetic mice show that dandelion extract may help regulate blood sugar and keep cholesterol in check (Pallab and Amartya, 2012).

2.11.8 Turmeric (*Curcuma longa)*

Curcuma longa belongs to the family Zingiberaceae and its rhizomes contain 5% volatile oil, resin; zingiberaceous starch grains and yellow coloured curcuminoids.

Turmeric possess hypoglycemic, hypolipidemic and antioxidant activity (Pallab and Amartya, 2012).

2.11.9 Bitter melon (*Momordica charantia*)

Bitter melon is widely cultivated in Asia, India, South America and East Africa for its intensely bitter fruits that are commonly used in cooking and as natural remedy for treating diabetes (Joseph and Jini, 2013). *Momordica charantia* belongs to the family Cucurbitaceae. It contains lectin that has insulin like activity due to its nonprotein specific linking together to insulin receptors. This lectin lowers blood glucose level by acting on peripheral tissues. The blood sugar lowering action of fresh juice or unripe fruit have been established in animal experimental models as well as human clinical trials (Chowdhury *et al.*, 2012).

2.11.10 Cinnamon (*Cinnamomum zeylanicum)*

Cinnamomum zeylanicum, belongs to the family Lauraceae and is used in the treatment of type II diabetes mellitus and insulin resistance. Alcoholic Extract of *Cinnamomum zeylanicum* leaves has shown antidiabetic Activity. Cinnamon improves glucose and lipid of people (Pallab and Amartya, 2012).

2.11 Plants of this Study

2.11.1 *Piper capense*

P. capense grows in moist, shaded places in forests and along streams (Palgrave, 2002). It is a shrubby herb 1-2m high, possibly sometimes subscandent, base semi-

woody, much branched above, stems glabrous. Leaf: petiole 2-6 cm long; leaf-blade broadly ovate. Flowers only slightly protandrous; stamens with anthers about as long as filaments; stigma on distinct style, 2-lobed. Drupe 4 mm in diameter, translucent greenish white, very fleshy (Edwards *et al.*, 2000). Fruits are made by little white closed berries of 8 cm. These berries turn to light green when it is harvest time. Dried fruit is ground up to produce pepper. However, it is unlikely that it is used for this purpose anymore because of the ready availability of pepper from the cultivated species *Piper nigrum* (Palgrave, 2002).

Figure 2.2: Fresh plant of *Piper capense*

2.11.2 *Berberis holstii*

A species of ericoid scrub of grassland or forest edges at 2-3000 m. Glabrous shrub up to 3 m tall; branches purplish when young, sulcate, with 3-partite spines up to 4 cm long. Leaves usually clustered on short lateral shoots, almost sessile, apparently simple but in reality 3-foliolate with a normal, terminal leaflet articulated at its base and a petiole bearing 2 subulate lateral leaflets 1-3 mm. long at its apex. The petiole is purplish when young and somewhat glaucous below (Wild *et al.,* 2004).

Flowers yellow, petals somewhat smaller than the inner sepals, Stamens on stout filaments; anthers, oblong, opening by a pair of spreading wing-like valves. Berry ellipsoid, dark blue, pruinose, with a persistent stigma and seeds usually solitary, ellipsoid, brown and rugulose (Wild *et al.*, 2004).

Berberis are medicinally used for various purposes (Srivastava *et al.,* 2006). Berberis are mostly used as ornamentals in Europe, and for medicinal purposes in many parts of the world. Barberries are sometimes used for preparing jams and dyes. Different alkaloids present in Berberis species makes them have therapeutic properties. These alkaloids include berberine, oxyacanthine, berbamine and palmatine (Srivastava *et al.,* 2006).

Figure 2. 3: Fresh plant of *Berberis holstii*

2.11.3 *Sonchus asper*

Sonchus asper is a dicotyledonous annual or biennial herb in the family Asteraceae (Grubben, 2004). This plant can grow up to 2 metres in height. It has a ridged, glabrous (hairless) central stem that is simple or branched and is pentagonal in cross section. The tap root is usually unbranched (Hutchinson *et al*., 1984). Young leaves

form a basal rosette, sometimes causing them to be confused with thistles. Mature leaves that occur on the flowering stem are alternate and have rounded lobes that clasp the stem. All leaves are glabrous, oblanceolate in shape, bluish green in colour and have prickly margins. Leaves and stems emit a milky sap (latex) when damaged (Huthchinson *et al.*, 1984).

Inflorescences are yellow in colour and occur in clusters at the end of stems (Hutchinson *et al*., 1984). Fruits are brown, wrinkled achenes. Achenes have 3 (or rarely 4-5) longitudinal ribs on ech face (Hutchinson *et al*., 1984). Mature seeds have a white feathery pappus (8mm long) that collectively form a white puff ball, similar to dandelion (Grubben and Denton, 2004).

Sonchus asper is eaten cooked and raw in salads (Grubben and Denton, 2004). The plant has been used to treat a vast variety of conditions, ailments and diseases including wounds, boils, asthma, bronchitis, gastrointestinal infections, malaria, venereal disease and many more. The latex has also been used to treat warts (Grubben and Denton, 2004). Chemical analysis shows that *S. asper* contains large quantities of phenolic compounds, flavonoids, ascorbic acid, carotenoids and a variety of other antioxidants.

Figure 2.4: Fresh plant of *Sonchus asper*

2.11.4 *Vernonia lasiopus*

The habitats of *V. lasiopus* are swamp forest margins and stream sides usually in swampy ground (Srivastava *et al.,* 2006). It is erect perennial herb or subshrub that may reach up to 2 m tall from a woody rootstock. Stems are erect, becoming woody below where leaves are petiolate, lanceolate to narrowly oblong-lanceolate sometimes ovate, apex tapering acute to obtuse, base cuneate to somewhat abruptly narrowed and attenuate down to the petiole, margins are coarsely serrate with callose-tipped teeth; upper surface drying dark-green, sparsely scabridulous-puberulous or glabrescent; lower surface pale greenish-grey (Srivastava *et al.,* 2006).

The plant is used to treat paralysis, epilepsy, convulsions, spasm and stomach troubles. Phytochemistry indicates that compounds presents are tannins and astringents. Roots are used to treat abortifacients and ecbolics (Preeth, 2013). In Tanganyika an extremely bitter decoction is made from the whole plant which is used for epilepsy,

indigestion and in childbirth. The root are also used to facilitate parturition (Preeth, 2013).

Figure 5: Fresh plant of *Vernonia lasiopus*

2.11.5 *Galinsoga parviflora*

G. parviflora is tough plant that will grow in sandy, loamy and clay soils. The herb is 2-7 m tall, sparsely pubescent with appressed or sometimes spreading hairs. Leaves are ovate or narrowly ovate, 2-5 cm long, 1-3 cm wide, and margins serrate or entire. Heads 3-4 mm high, peduncles appressed pubescent or glandular villous; involucral bracts 2-3 mm long; ray florets 3-6 per head, rays white, rarely pink, 3-toothed, 1-2 mm long; pappus of ray florets absent or very reduced, that of disk florets consisting of blunt-tipped, fimbriate scales. Achene is sparsely appressed pubescent or glabrous (Wild *et al.*, 2004).

The leaves stem and flowering shoots raw or cooked are eaten as a potherb, or added to soups and stews. They can be dried and ground into a powder then used as flavoring in soups. A bland but very acceptable food, it makes a fine salad either on its own or mixed with other leaves. The fresh juice can be mixed and drunk with tomato or

vegetable juices. When rubbed onto the body, the plant is useful in treating nettle stings. The juice of the plant is applied to treat wounds; it helps to coagulate the blood of fresh cuts and wounds (Wild *et al.*, 2004).

Figure 2.6: Fresh plant of *Galinsoga parviflora*

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Site

This study was done at the Department of Biochemistry and Biotechnology Animal House in School of Pure and Applied Sciences of Kenyatta University in 2014.

3.2 Collection and preparation of plant materials

Fresh roots of *Berberis holstii*, *Piper capense* and fresh leaves of *Sonchus asper*, *Vernonia lasiopus* and *Galinsoga parviflora* was collected from their natural habitat in Gilgil District of Nakuru County, Kenya. The collection was done on basis of the reports given by local herbalists on hypoglycemic activity of the plants. The plants were identified and authenticated at the National Museum of Kenya herbarium. The collected plants parts were cut into small pieces. The roots and leaves were air-dried away from direct sunlight for four weeks. The dried materials were then crushed into powder using an electric mill.

3.3 Extraction

One hundred grams of powdered plant leaves and roots were extracted in one litre of water at 60^{o}C for 6 hours. The extract was left to cool and then decanted through folded cotton gauze. The filtrate was freeze-dried in 200 ml portions for 48 hours. The concentrated extract was weighed and stored in air-tight sample bottle at -20^{o}C until used for bioassay.

3.4 Determination of hypoglycemic activities

3.4.1 Experimental Animals

Male White Albino mice aged 3-4 weeks and weighing 22-27g were used.. The mice were housed and fed on rodent pellets and water *ad libitum*.

3.4.2 Induction of diabetes

The animals were fasted for at least 8 hours but were given water until the end of this experiment. Diabetic state was induced by an intraperitoneal administration at a dose of 186.9 mg/kg body weight of 10% alloxan monohydrate. Two days after induction of hyperglycemia, a glucometer was used to measure blood sugar and only mice with blood glucose > 2000 mg/L or >11.1 mmol/L were considered diabetic and used in the study (Szkudelski, 2001).

3.4.3 Experimental design

For each plant, the albino mice used in this study were divided into eight groups of five animals each. Group I mice (Normal control) were normal and were orally administered with 0.1 ml of physiological saline. Group II mice (Negative control) were diabetic and were orally administered with 0.1 ml physiological saline. Group III mice (Positive control) were diabetic and were orally administered with Glibenclamide (reference drug) at a dose of 200mg/kg body weight. Group IV, V, VI, VII, and VIII mice (Experimental groups) were diabetic and were orally given the plant extracts at doses of 25, 48.4, 93.5, 180.9, and 350 mg/kg body weight, respectively. This design was repeated but the treatment was administered

intraperitoneally. The positive controls for intraperitoneal route were administered with 0.12 units of insulin, the reference drug (1 IU/kg body weight) in 0.1ml saline.

3.5 Blood Sampling and Blood Glucose, Rate constant and Half-Life Determination

Imediately after administering the mice with the extract, blood samples were taken and this was at 0 hours. Sampling of blood was done by sterilizing the tail with 10% alcohol and then nipping it and that was repeated after 2, 4, 6, 8, 10 and 24 hours. Small amount of blood was then squeezed into a Glucometer. The percentage glucose reduction at each time point was calculated using the formula below:

$$\text{Percentage reduction in glucose} = \frac{\text{Glucose concentration at a given time}}{\text{Glucose concentration at 0 hour}} \times 100$$

The rate constant (k) was obtained by plotting log concentration of blood glucose for the first four hours against time in hours. This gave the pseudo-first order rate constant (k/2.303) with a constant indicating the point where the straight line intersects the natural logarithm of glucose concentration axis (indicating the original glucose concentration before the drug administration) (Lin and Cadenas, 2003). The half-life was calculated by substituting for the rate constant (k) in the formulae: $t_{0.5} = 0.693/k$ whereby $t_{0.5}$ is the time when the dosage reduces the plasma sugar level by half (John, 1989) cited by Piero et al., 2015. The exponential decay equation was used to get the dosage that would be administered after a certain period (Evan *et al.*, 1992).

3.6 Preliminary *in vivo* toxicity analysis

Six groups of normal albino mice (five animals each) were used for preliminary *in vivo* toxicity analysis. Group I was treated with 0.1ml physiological saline for 28 days and served as control. The rest of the groups were, each treated either orally or intraperitoneally with 10.4 mg, 15.4 mg and 23 mg (450, 670 and 1000 mg/kg body weight, respectively) of the extract of *Piper capense, Berberis holstii, Sonchus asper, Vernonia lasiopus,* and *Galinsoga parviflora* in 0.1ml physiological saline. The animals were orally or intraperitoneally administered with the extracts daily for 28 days and kept under close observation and fed on standard mice pellets and water. Observations were made on animals for signs of acute toxicity, morbidity and mortality. Any animal that died or showed signs of death were sacrificed. The animals that were still alive after 28 days were sacrificed for determination of hematological parameters, biochemical parameters and organ weight.

3.6.1 Determination of Body and Organ Weight

Body weight of mice was determined weekly during the dosing period for 28 days. All the animals were euthanized and sacrificed on the 28^{th} day. The liver, heart, lungs, kidneys, spleen, testis and brain were dissected out, weighed and then stored in 10% neutral buffered formalin. The relative organ to body weight was then calculated using the formular below:

Relative organ to body weight

= [Weight of the organ/Individual mice weight on day of sacrifice] × 100

3.6.2 Determination of Hematological Parameters

Blood parameters were determined using standard protocols (Jain, 1986) as cited by Piero *et al.,* (2015). White blood cells (WBC), red blood cells (RBC), mean cell hemoglobin (MCH), hemoglobin (Hb), mean cell volume (MCV) and mean cell hemoglobin concentration (MCHC) were determined using the Coulter Counter System. Differential white blood cell count for monocytes (MON), eosinophils (EOS), lymphocytes (LYMP), neutrophils (NEU), and basophils (BAS) were determined using stained blood films by a hemocytometer. Blood films were dried in air and stained with Giemsa stain then examined microscopically using a magnification of X400 and X1000 for cell morphologies and differential WBC counts, respectively. The rest of blood was collected in tubes (plastics), left for three hours for clotting to occur. Clotted samples were centrifuged for ten minutes to obtain clear samples of serum which were stored frozen at -20^0C until biochemical parameter analysis was done.

3.6.3 Determination of Biochemical Parameters

Biochemical parameters determined on the sera specimen were LDH (lactate dehydrogenase), AST (aspartate aminotransferase), ALT (alanine aminotransferase) GGT (γ-glutamyltransferase), ALP (alkaline phosphatase), BUN (blood urea nitrogen), CREAT (creatinine), AMY (amylase) and CK (creatinine kinase).

3.6.3.1 Determination of Serum Levels of Aspartate Aminotransferase (AST)

Aspartate aminotransferase (AST) was determined using the method described by Henry *et al.* (1960). AST reagent was used to measure AST level by an enzymatic rate method. In the reaction, AST catalyzed the reversible transamination of L-aspartate and α-ketoglutarate to oxaloacetate and L-glutamate. The oxaloacetate was then reduced to malate in the presence of malate dehydrogenase (MDH) with concurrent oxidation of reduced β-nicotinamide adenine dinucleotide (NAD). The ratio of the sample to reagent was 1 part sample to 11 parts reagents (23μL: 253μL reagent). The absorbency was measured at 340 nm and its change was directly proportional to the levels of AST. The machine calculated and expressed the level in IU/L. The reaction took place at 37^0C for three minutes. The principal of the reaction is as follows:

2-oxoglutarate + L-aspartate $\xleftrightarrow{AST}$ L-glutamate + Oxaloacete

Oxaloacetate + NADH + H+ $\xleftrightarrow{MDH}$ Malate + NAD^+

3.6.3.2 Determination of Serum Levels of Alanine Aminotransferase (ALT)

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

Pyridoxal-5-phosphate was required in this reaction as a cofactor for the transaminase activity by binding to the enzyme using Schiff-base linkage. The ratio of the sample to reagent was 1 part sample to 11 parts reagents (23 μL: 253μL reagent). The absorbance was measured at 340 nm and its change was directly proportional to the level of ALT. The machine calculated and expressed the level in IU/L. The reaction took place at 37^0C for 3min. The principal of the reaction is as follows:

2-oxoglutarate + L-alanine $\xleftrightarrow{ALT}$ L-glutamate + pyruvate

Pyruvate + NADH + H^+ $\xleftrightarrow{LDH}$ Lactate + NAD^+

3.6.3.3 Determination of Serum Levels of Alkaline Phosphatase (ALP)

The ALP reagent was used to measure ALP level by kinetic method using a 2-amino-2-methyl-1-propanol (AMP) buffer. In the reaction, ALP catalyzed the hydrolysis of the colorless organic phosphate ester substrate (p-nitrophenylphosphate) to the yellow colored product (p-nitrophenol and phosphate). This reaction occurred at alkaline pH of 10.3. The ratio of the sample to reagent was 1 part sample to 50 parts reagents (5μL: 250μL reagent). The absorbance was measured at 410 nm and this change was directly proportional to the level of ALP. The machine calculated and expressed the level in IU/L. The reaction took place at 37^0C for three minutes (International Federation of Clinical Chemistry, 1983). The principal of the reaction is as follows:

4-NPP + H_2O $\xrightarrow{ALP}$ 4-NPO + Phosphate

3.6.3.4 Determination of Serum Levels of γ-glutamyl Transferase (GGT)

GGT reagent was used to measure γ-glutamyltransferase level by an enzymatic kinetic UV rate method. In the reaction, γ-glutamyltransferase catalyzed the transfer of the glutamyl group from the substrate to glycylglycine forming glutamylglycylglycine and 5-amino-2-nitrobenzoate. 5µl of the sample was reacted with 200µl of the reagent. The rate of formation of 5-amino-2-nitrobenzoate was proportional to the level of GGT present in the sample and was measured kinetically at 405nm. The level was calculated and expressed in U/L. The reaction took place at 37^{o}C for three minutes. The principal of the reaction is as follows:

L-γ–glutamyl-3-carboxy-4-nitroanilide + glycylglycine $\xrightarrow{GGT}$ L-γ-glutamylglycylglycine + 5-amino-2-nitrobenzoate

3.6.3.5 Determination of Serum Levels of Lactate Dehydrogenase (LDH)

Lactate dehydrogenase (LDH) was determined using an enzymatic kinetic UV test. In the reaction, LDH catalyzed the oxidation of lactate to pyruvate coupled with the reduction of NAD^+ to NADH. 2 µL of sample was reacted with 40 µL of reagent and the change in absorbance due to reduction of NAD was monitored at 340nm. This change was directly proportional to the concentration of LDH in the sample and was used to calculate and express concentration in U/L. The reaction took place at 37^{o}C for three and half minutes. The pH optimum for lactate –to- pyruvate (L $\longrightarrow$ P) reaction is 8.8 to 9.8. The principal of the reaction is as follows:

Lactate + NAD^+ $\xrightarrow{LDH}$ Pyruvate + NADH + H^+

3.6.3.6 Determination of Serum Levels of Creatine Kinase (CK)

Creatine kinase (CK) was determined using the method described Oliver and Rosalki which is based on the primary reaction that is catalyzed by CK resulting in production of creatine and ATP. The ATP produced in the primary reaction is then employed in a coupled enzymatic glucose assay employing hexokinase (HK) and glucose-6-phosphate dehydrogenase (G6PD). The ratio of the sample to reagent was 1 part sample to 50 parts reagents (5µL: 250µL reagent). The machine calculated and expressed the level in IU/L. The reaction took place at 37^0C for three minutes. The production of NADPH in the indicator reaction is monitored at 340 nm and is related to CK level within the patient specimen (Rosalki, 1967). The principal of the reaction is as follows:

$$\text{ADP + Creatinine phosphate} \xrightarrow{CK} \text{Creatinine + ATP}$$

$$\text{ATP + Glucose} \xrightarrow{HK} \text{ADP + Glucose-6-phophate}$$

$$\text{Glucose-6-phophate+NAD}^+ \xrightarrow{G6PD} \text{6-Phosphogluconate + NADH + H}^+$$

3.6.3.7 Determination of Serum Levels of α-amylase (AMY)

Amylase reagent was used to measure the concentration of amylase by a kinetic colour method using Olympus Autoanalyzer. In the reaction, 2-chloro-4-nitrophenyl-α-D-maltotrioside ($CNPG_3$) substrate reacted with amylase in the serum to release 2-chloro-4-nitrophenol (CNP) from the substrate, which was directly proportional to the concentration of amylase in the sample. Three (3) µL of sample was reacted with 300 µL of reagent and the change in absorbance was monitored at 340nm, due to reduction of NAD.

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

$$CNPG_3 + H_2O \xrightarrow{Amylase} CNP + Maltotriose$$

3.6.3.8 Determination of Serum Levels of Total Bilirubin (T-BIL)

Total bilirubin reagent was used to measure BIL-T concentration by timed end point reaction. In the presence of hydrochloric acid (HCL), T-BIL was coupled with diazotized Sulfanilic acid (pH 1.4) forming azobilirubin. The chemistry analyzer automatically aliquoted 9 µl sample and 120 µl BIL-T reagents then photometrically measured azobilirubin whose colour intensity was directly proportional to the T-BIL concentration. Determination took place at 552 nm for two minutes at 37^{0}C. The analyzer automatically calculated the T-BIL concentration and expressed it in µmol/L. The principal of the reaction is as follows:

$$\text{Sulfanilic acid} + NaNO_2 \xrightarrow{HCl} \text{Diazotized Sulfanilic acid}$$

$$\text{Bilirubin} + \text{Diazotized Sulfanilic acid} \xrightarrow{pH\,1.4} \text{Azobilirubin}$$

3.6.3.9 Determination of Serum Levels of Direct Bilirubin (D-BIL)

Conjugated and direct bilirubin reagent was used to measure D-BIL concentration by timed end point reaction. In the presence of hydrochloric acid (HCl), D-BIL was coupled with diazotized Sulfanilic acid (pH 1.4) forming azobilirubin. The chemistry analyzer automatically aliquoted 9 µl sample and 120µl D-BIL reagent then photometrically measured azobilirubin whose colour intensity was directly

proportional to the D-BIL concentration. Determination took place at 552 nm for two minutes at $37^{O}C$. The analyzer automatically calculated the D-BIL concentration and expressed it in µmol/L. The principal of the reaction is as follows:

Sulfanilic acid + $NaNO_2 \xrightarrow{HCl}$ Diazotized Sulfanilic acid

Bilirubin + Diazotized Sulfanilic acid $\xrightarrow{pH1.4}$ Azobilirubin

3.6.3.10 Determination of Serum Levels of Urea

Blood urea nitrogen (BUN) was determined using the method described by Tiffany *et al* (1972). The BUN reagent was used to measure the concentration of urea by an enzymatic rate method. In the reaction, urea was hydrolyzed by urease to ammonia and carbon dioxide. Glutamate dehydrogenase (GlDH) catalyzed the condensation of ammonia and α-ketoglutarate to glutamate with concomitant oxidation of reduced β-Nicotinamide Adenine Dinucleotide (NADH) to β-Nicotinamide Adenine Dinucleotide (NAD^{+}). The ratio of the sample to reagent was 1 part sample to 100 parts reagents (3µL: 300µL reagent). The absorbance was measured at 340 nm and this change was directly proportional to the concentration urea. The machine calculated and expressed the concentration in µM. The reaction took place at $37^{0}C$ for one minute. The principal of the reaction is as follows:

Urea + $H_2O \xrightarrow{Urease}$ $2NH_3 + CO_2$

NH_3 + α-ketoglutarate + NADH $\xrightarrow{GlDH}$ L-Glutamate + NAD^{+}

3.6.3.11 Determination of Serum Levels of Creatinine (CREAT)

Creatinine reagent was used to measure CREAT concentration by a modified rate Jaffe. In the reaction, creatinine reacted with picric acid in alkaline solution to form creatinine-picric acid complex. The analyzer automatically proportioned 15 µl sample and 250 µl creatinine reagent into the reaction cuvette. The rate of colour formation was monitored and increase in absorbance determined at 512 nm for two minutes at 37^0C. The analyzer automatically calculated the CREAT concentration and expressed it in mmol/L. The principal of the reaction is as follows:

Creatinine + Picric acid $\xrightarrow{pH13}$ Creatinine-picric acid (yellow-red) complex

3.7 Quantitative Phytochemical Screening

Phytochemical screening for alkaloids, saponins, flavonoids, total phenols and tannins in *Piper capense, Berberis holstii, Sonchus asper, Vernonia lasiopus,* and *Galinsoga parviflora* extracts were performed using standard methods (Houghton and Raman, 1998). For each of the phytochemicals, screening was done as follows:

3.7.1 Saponins

Saponins quantification was done according to Obadoni and Ochuko method (2001) with modifications. Extraction of 2.0 g of each sample with methanol was done in Soxhlet apparatus for 8 hours. Evaporation of the extract was done under reduced pressure and the crude extracts partitioned between hexane and water in separating funnels. Diethyl ether was then used to extract aqueous layers. Diethyl ether layers were discarded to recover the aqueous layer and the purification process repeated.

Partitioning of aqueous layers was done with butanol 3 times. Extracts washing was done two times with 5 percent sodium chloride and then evaporated to produce crude saponins with the contents being expressed as percentage.

3.7.2 Alkaloids

The gravimetrical method was used to determine content of alkaloid (Harbone, 1973) with modifications. 1.0 g of each sample was defatted 3 times in hexane. 50 ml of 10% acetic acid in ethanol was then used to extract. Obtained product was shaken well, and left to stand for four hours. It was then filtered followed by concentration in water bath. Concentrated ammonium hydroxide was added drop by drop to precipitate the alkaloids. Precipitates were filtered using filter papers then washed with one percent ammonium hydroxide. Drying of precipitates was done in an oven at $60^{o}C$ for thirty minutes and transferred into desiccator for cooling to a constant weight. Duplication of the experiments was done for each sample and average reading was recorded.

3.7.3 Total flavonoid

Concentration of flavonoid was determined using aluminium chloride colorimetric assay (Marinova *et al.,* 2005). 0.1 g of the extract was added to 4 mL of double distilled water. 0.3 mL of 5% $NaNO_2$ was added to the resulting mixture followed by 0.3 mL of 10% $AlCl_3$ after 5 minutes. This was followed by addition of 2 mL of 1 M sodium hydroxide and the volume made up to 10 mL with double distilled water. Measurement of absorbance was then done at 510 nm. Quercetin was used as standard to measure the flavonoid content.

3.7.4 Tannins

Tannins determination was done as follows; 0.1g of each sample was extracted three times in 70% acetone. The sample was centrifuged and supernatant removed. Aliquots were taken and final volume adjusted to 3 ml using distilled water. Vortexing of the solution was done followed by addition of 1 ml of 0.016M $K_3Fe(CN)_6$, then 1 ml of 0.02 M $FeCl_3$ in 0.10 M HCl. Vortexing was repeated and the tubes were kept as such for 15 min. 5 ml of stabilizer was added followed by revortexing. Measurement of absorbance was done at 725 nm. Plotting of standard curve was done using various concentrations of tannic acid (Gurib-Fakim, 2006).

3.7.5 Total phenols

Folin-Ciocalteau reagent and tannic acid as standard ware used to determine phenolic total content according to the method by Rasineni *et al*., (2008). 100 mg of sample was homogenized in 10ml of 70% acetone. Centrifuging of homogenate was done at 10,000 × g for twenty minutes. Total phenols were determined from supernatant as follows. To 2.5 ml of the supernatant 0.5 ml of Folin-Ciocalteau was added and then 2 ml of 10% sodium carbonate in ethanol. Incubation of the mixture was done for 5 minutes at 20ºC and then reading of absorbance done in triplicates at 750 nm wavelengths.

3.8 Mineral Composition of Plant extracts

The total reflection X-ray fluorescence (TRXF) system were used to determine the concentration of copper, lead, iron, manganese, rubidium, calcium, potassium, zinc,

and bromine in the leaf extracts of *Piper capense, Berberis holstii, Sonchus asper, Vernonia lasiopus,* and *Galinsoga parviflora*. The Total Reflection X-ray Fluorescence System analysis consists of an X-ray spectrometer and a radioisotope excitation source

3.8.1 Sample Preparation

1 g each (3 sets) of lyophilized sample were weighed into clean vials. 10mL of double distilled water was added to each sample for dissolution. 20µL of stock solution was added into each sample (as internal standard). The samples were homogenized for 1 minute using a mixer. Portions of 10µL of each sample were pipetted out using a micropipette onto a clean quartz carrier. The samples were then dried to evaporate the liquid.

3.8.2 TXRF system

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

3.8.3 Sample Spectrum Acquisition and Quantitative Analysis

Sample carriers were irradiated for 1000 seconds using Spectrometer. Evaluation of the measured spectra was done using software on the basis of the chosen elements.

Using the same software, concentrations were determined based on the intensities of the element peaks according to the following formula:

$$C_x = \frac{Nx / Sx}{Nis / Sis} xCis$$

Where, C_x = Concentration of the analyte

C_{is} = Concentration of the internal standard

N_x= Net intensity of the analyte

N_{is}= Net intensity of the internal standard

S_x= Relative sensitivity of analyte

S_{is}= Relative sensitivity of internal standard

3.8.4 Atomic Absorption Spectrophotometry (AAS)

Analysis of Cadmium, Chromium and Magnesium was performed using Atomic Absorption Spectrophotometry. Samples and standard solutions were prepared as described by Piero *et al*. (2012e) with slight modifications. Enough amounts of standard stock solutions of each element were taken and diluted to volume and transferred into plastic beakers. Standard solutions for each element were prepared within a given range. Standard blanks for each element were prepared. No sample digestion was required as the freeze dried aqueous extracts of the plant samples were used.

The instrument was set to the right conditions for each element, and the respective standard and sample solutions were aspirated into the flame one at a time to determine their absorbance. Water was flushed into the flame to establish the zero absorbance.

Procedure was repeated two times for each sample and element. Calibration curve for each element was prepared by plotting a graph of mean absorbance against corresponding concentrations of the standard solutions. Microsoft Excel computer software was used to convert absorbance readings to concentrations of element. The results ware expressed in ppm≈µg/g (parts per million).

3.9 Data Management and Statistical Analysis

Collected data was entered in Excel, cleaned and then exported to SPSS software for analysis. Results were expressed as Mean ± standard deviation (SD) of the number of animals used per every study point. Statistical analysis was done using ANOVA and post-ANOVA (Bonferroni-Holm) to compare the means of untreated normal control mice with diabetic mice treated with saline, diabetic mice treated with the conventional drug, diabetic mice treated with plant extracts at doses of 25, 48.4, 93.5, 180.9, and 350 mg/kg body weight. $\rho \leq 0.05$ was considered statistically significant.

CHAPTER FOUR

RESULTS

4.1 Effects of administration of extracs on blood glucose levels

4.1.1 Effects of administration of *Berberis holstii* extracts on blood glucose levels in alloxan induced diabetic mice

Berberis holstii root extract yielded a dark brown lyophilate at a concentration of 62.5 mg/g dry weight. Oral administration of aqueous extracts of *Berberis holstii* at 25, 48.4, 93.5, 180.9, and 350 mg/kg body weight to diabetic mice decreased the blood glucose levels significantly as from the 2^{nd} hour independent of the dose. The percentage reductions of blood glucose levels in mice by the aqueous extract of *Berberis holstii* at the five dose levels (25, 48.4, 93.5, 180.9, and 350 mg/kg body weight) during the 2^{nd} hour were 85.0, 66.3, 65.2, 73.7 and 77.5%, respectively, compared to glibenclamide treated diabetic mice whose blood sugar levels was lowered to 78.1% within the same hour (Figure 4.1). At this hour the plant extract lowered blood sugar levels but not to normal ($^{d}\rho < 0.05$) (Table 4.1). However, the 93.5mg/kg body weight dose, lowered blood sugar levels significantly in comparison to diabetic control ($^{a}\rho < 0.05$) (Table 4.1).

In the 4^{th} hour, the glucose lowering effect by the five dose levels was also observed, as the percentage reduction of blood glucose was 69.6, 43.6, 49.9 55.6 and 59.8%, respectively, compared to glibenclamide treated diabetic mice whose blood sugar levels was lowered to 57.7% within that hour. In the 6^{th} hour, the five tested doses of the aqueous extract of *B. holstii* lowered blood sugar levels to 51.3, 35.8, 39.0, 40.2 and 46.8%, respectively, compared to glibenclamide treated diabetic mice whose

blood sugar levels was lowered to 41.9%. At this hour, the blood glucose levels in diabetic mice were lowered to those of the normal by the five extract doses ($^{d}\rho > 0.05$). Further, at this hour, the extract lowered blood glucose levels as effectively as glibenclamide ($^{b}\rho < 0.05$) especially by the 180.9 mg/kg body weight. The same trend was observed during the 8th hour, where the five dose levels lowered blood glucose to levels lower than that of glibenclamide (37.0%). During this hour, the percentage reduction in blood glucose level in diabetic mice treated with the five aqueous extract doses was 41.5, 30, 33.9, 38.7 and 33.9%, respectively (Figure 4.1). All the diabetic mice mice treated with the five aqueous extracts doses had returned to the diabetic state at the 24th hour.

Intraperitoneal administration of aqueous extracts of *Berberis holstii* at 25, 48.4, 93.5, 180.9, and 350 mg/kg body weight to mice decreased the blood glucose levels significantly as from the 2nd hour independent of the dose. During the 2nd hour, the percentage reduction of blood glucose levels by the aqueous extract of *Berberis holstii* at 25, 48.4, 93.5, 180.9, and 350 mg/kg body weight was 72.7, 63.4, 57.5, 67.6 and 73.2%, respectively, compared to insulin treated diabetic mice whose blood sugar levels was lowered to 50.5% within the same hour (Figure 4.2). At this hour, the aqueous plant extract lowered the blood glucose levels in diabetic mice but not to levels of normal mice ($^{C}\rho < 0.05$) (Table 4.1). However, the blood glucose level in the diabetic mice treated with the five aqueous extracts doses was significantly lowered relative to that of the diabetic control mice ($^{A}\rho < 0.05$) (Table 4.1).

During the 4th hour, the glucose lowering effect by the five dose levels was also observed; the percentage reduction of blood glucose was 58.5, 44.9, 41.9, 53.7 and 53.6%, respectively, compared to insulin treated diabetic mice whose blood sugar levels was lowered to 47.4% within that hour. By the 6th hour, the aqueous extract of *B. holstii* doses lowered blood sugar levels to 47.3, 36.4, 34.3, 40.0 and 43.5%, respectively, compared to insulin treated diabetic mice whose blood sugar levels was lowered to 41.2%. The five aqueous extract lowered blood glucose levels to normal ($^{C}\rho > 0.05$). Further, at this hour, the five aqueous extracts doses lowered blood glucose levels as effectively as insulin ($^{B}\rho < 0.05$) especially the 180.9 mg/kg body weight dose. During the 8th hour, the same trend in blood glucose reduction in diabetic mice was observed where the five dose levels lowered blood glucose levels to levels lower than insulin, which lowered blood glucose level to 38.1%. The percentage reduction of blood glucose by the intraperitoneal route was 40.1, 31.5, 30.6, 34.5 and 33.2%, respectively (Figure 4.2). At the 24th hour, the blood glucose reduction in diabetic mice by all the five extracts doses had returned to that of the diabetic control mice.

Intraperitoneal administration of aqueous extracts of *Berberis holstii* at 25 mg/kg body weight was more effective in lowering blood glucose levels in diabetic mice than the oral route. The rest of the extracts doses lowered blood glucose in diabetic mice to similar levels regardless of the route of administration (Table 4.1).

Table 4.1: Effects of administration of five therapeutic doses of aqueous extracts of *Berberis holstii* at different times on blood glucose levels in alloxan induced diabetic mice

Treatment	Route	Levels of Glucose at Varying Times (mmol/L)					
		0 hour	2 hour	4 hour	6 hour	8 hour	24 hour
Normal control	IP	5.20±0.03 ^{ABDEFGH}	5.22±0.04^{ABDEH}	5.22±0.05^{A}	5.18±0.04^{A}	5.22±0.04^{A}	5.20±0.01^{ADEFG}
	Oral	5.22±0.04 ^{abefghi}	5.22±0.04 ^{ae}	5.20±0.05^{ae}	5.16±0.04^{a}	5.18±0.02^{a}	5.24±0.04^{ae}
Negative control	IP	14.02±1.16^{C}	15.34±1.22^{CBDEFG}	16.33±1.31^{CBDEFGH}	18.06±1.31^{CBDEFGH}	19.34±1.32^{CBDEFGH}	22.94±1.49^{CBDEFGH}
	Oral	15.64±1.71^{d}	16.60±1.74^{bei}	18.10±1.61	19.28±1.79	20.64±1.65	23.16±1.81
Positive control (insulin)	IP	13.12±0.97^{C}	6.62±0.16^{AH}	6.22±0.12^{A}	5.40±0.10^{A}	5.00±0.07^{A}	6.78±0.21^{A}
Positive control (glibenclamide)	Oral	13.4±0.86^{d}	10.56±0.53^{d}	7.20±0.37^{a}	5.62±0.28^{a}	4.96±0.08^{a}	8.04±0.42^{a}
Extract dose (mg/kg body weight)							
25	IP	14.22±1.03^{C}	10.34±0.79^{CA*}	8.32±0.64^{A*}	6.72±0.55^{A*}	5.70±0.25^{A*}	10.44±0.70^{C}
	Oral	15.32±1.72^{d}	13.20±1.98^{d*}	10.80±1.75^{da*}	7.96±1.33^{a*}	6.44±0.68^{a*}	12.30±1.61^{da}
48.4	IP	15.42±1.33^{C}	9.78±1.17^{CA}	6.92±0.70^{A}	5.56±0.28^{A}	4.86±0.18^{A}	9.34±0.90^{CA}
	Oral	15.60±2.28^{d}	10.34±1.39^{d}	6.80±0.61^{a}	5.58±0.48^{a}	4.68±0.27^{a}	10.12±1.07^{a}
93.5	IP	14.46±0.82^{C}	8.32±0.71^{A}	6.06±0.42^{A}	4.96±0.09^{A}	4.42±0.15^{A}	9.34±0.49^{CA}
	Oral	14.32±1.38^{d}	9.34±1.44^{a}	6.86±0.94^{a}	5.58±0.56^{a}	4.86±0.46^{a}	10.32±0.87^{a}
180.9	IP	13.70±1.78^{C}	9.26±0.76^{A}	7.36±0.62^{A}	5.48±0.50^{A}	4.72±0.40^{A}	9.48±0.86^{CA}
	Oral	14.22±1.63^{d}	10.48±1.20^{d}	7.90±0.76^{a}	5.72±0.55^{a}	5.5.±0.66^{a}	9.90±0.90^{a}
350	IP	15.50±1.37^{C}	11.36±1.41^{CB}	8.30±0.66^{A}	6.74±0.39^{A}	5.14±0.12^{A}	7.14±0.25^{A}
	Oral	16.36±1.71^{d}	12.68±1.70^{d}	9.78±1.45^{a}	7.66±0.84^{a}	5.54±0.47^{a}	7.24±0.96^{a}

Results are expressed as Means ± Standard Deviation for five mice per group. Means accompanied by similar upper case letters and similar lower case letters in the same column are significantly different at $\rho \leq 0.05$ by ANOVA and post ANOVA (Bonferroni-Holm) test. Means for IP administration: $^{C}\rho < 0.05$ with respect to normal control; $^{A}\rho < 0.05$ with respect to negative control; $^{B}\rho < 0.05$ with respect to positive control; $^{D}\rho < 0.05$ with respect to 25 mg/kg body weight; $^{E}\rho < 0.05$ with respect to 48.4 mg/kg body weight; $^{F}\rho < 0.05$ with respect to 93.5 mg/kg body weight; $^{G}\rho < 0.05$ with respect to 180 mg/kg body weight; $^{H}\rho < 0.05$ with respect to 350 mg/kg body weight. Means for Oral administration: $^{d}\rho < 0.05$ with respect to normal control; $^{a}\rho < 0.05$ with respect to negative control; $^{b}\rho < 0.05$ with respect to positive control; $^{e}\rho < 0.05$ with respect to 25 mg/kg body weight; $^{f}\rho < 0.05$ with respect to 48.4 mg/kg body weight; $^{g}\rho < 0.05$ with respect to 93.5 mg/kg body weight; $^{h}\rho < 0.05$ with respect to 180 mg/kg body weight; $^{i}\rho < 0.05$ with respect to 350 mg/kg body weight. Value followed by $*\rho < 0.05$ is considered statistically significant when the mean of the oral group is compared to intraperitoneal group by T-test.

Table 4.2: Effects of administration of five therapeutic doses of aqueous extracts of *Berberis holstii* at different times on blood glucose levels in alloxan induced diabetic mice

Treatment	Route	Levels of Glucose at Varying Times (mmol/L)					
		0 hour	2 hour	4 hour	6 hour	8 hour	24 hour
Normal control	IP	5.20±0.03	5.22±0.04	5.22±0.05	5.18±0.04	5.22±0.04	5.20±0.01
	Oral	5.22±0.04	5.22±0.04	5.20±0.05	5.16±0.04	5.18±0.02	5.24±0.04
Negative control	IP	14.02±1.16^{kmnop}	15.34±1.22^{kmnop}	16.33±1.31^{kmnop}	18.06±1.31^{kmnopq}	19.34±1.32^{kmnopq}	22.94±1.49^{oq}
	Oral	15.64±1.71^{JLNQR}	16.60±1.74^{JLNQR}	18.10±1.61^{JLNQR}	19.28±1.79^{JLNQRS}	20.64±1.65^{JLNQRS}	23.16±1.81^{QRS}
Positive control (insulin)	IP	13.12±0.97	6.62±0.16^{mnopq}	6.22±0.12^{mnopq}	5.40±0.10^{mnopq}	5.00±0.07^{mnopq}	6.78±0.21^{mnopq}
Positive control (glibenclamide)	Oral	13.40±0.86	10.56±0.53	7.20±0.37^{NS}	5.62±0.28^{QR}	4.96±0.08^{QR}	8.04±0.42^{NS}
Extract dose (mg/kg body weight)							
25	IP	14.22±1.03	10.34±0.79^{mnq}	8.32±0.64^{mnopq}	6.72±0.55^{nop}	5.70±0.25^{nop}	10.44±0.70^{mnq}
	Oral	15.32±1.72^{JLS}	13.20±1.98^{JLNS}	10.80±1.75^{LNQRS}	7.96±1.33^{NQRS}	6.44±0.68^{NQR}	12.30±1.61^{JLNQS}
48.4	IP	15.42±1.33	9.78±1.17^{mnq}	6.92±0.70^{mnopq}	5.56±0.28^{nopq}	4.86±0.18^{nop}	9.34±0.90^{mnoq}
	Oral	15.60±2.28	10.34±1.39^{LNS}	6.80±0.61^{LNQRS}	5.58±0.48^{NQR}	4.68±0.27^{NQR}	10.12±1.07^{LNS}
93.5	IP	14.46±0.82	8.32±0.71^{mnq}	6.06±0.42^{mnop}	4.96±0.09^{nop}	4.42±0.15^{nop}	9.34±0.49^{mq}
	Oral	14.32±1.38	9.34±1.44^{LNS}	6.86±0.94^{LNQR}	5.58±0.56^{NQR}	4.86±0.46^{NQR}	10.32±0.87^{LS}
180.9	IP	13.70±1.78	9.26±0.76^{mnq}	7.36±0.62^{mnopq}	5.48±0.50^{nop}	4.72±0.40^{nop}	9.48±0.86^{mnq}
	Oral	14.22±1.63	10.48±1.20^{LNS}	7.90±0.76^{LNQRS}	5.72±0.55^{NQR}	5.5.±0.66^{NQR}	9.90±0.90^{LNS}
350	IP	15.50±1.37	11.36±1.41^{mn}	8.30±0.66^{mnopq}	6.74±0.39^{nopq}	5.14±0.12^{nopq}	7.14±0.25^{nopq}
	Oral	16.36±1.71	12.68±1.70^{LN}	9.78±1.45^{LNQS}	7.66±0.84^{NQRS}	5.54±0.47^{QRS}	7.24±0.96^{NQRS}

Results are expressed as Means ± Standard Deviation for five mice per group. Means accompanied by similar upper case letters and similar lower case letters in the same row are not significantly different at $\rho \leq 0.05$ by ANOVA and post ANOVA (Bonferroni-Holm) test. Means for IP administration: $^{k}\rho < 0.05$ with respect to 0 hr; $^{m}\rho < 0.05$ with respect to 2 hr; $^{n}\rho < 0.05$ with respect to 4 hr; $^{o}\rho < 0.05$ with respect to 6 hr; $^{p}\rho < 0.05$ with respect to 8 hr; $^{q}\rho < 0.05$ with respect to 24 hr. Means for Oral administration: $^{J}\rho < 0.05$ with respect to 0 hr; $^{L}\rho < 0.05$ with respect to 2 hr; $^{N}\rho < 0.05$ with respect to 4 hr; $^{Q}\rho < 0.05$ with respect to 6 hr; $^{R}\rho < 0.05$ with respect to 8 hr; $^{S}\rho < 0.05$ with respect to 24 hr.

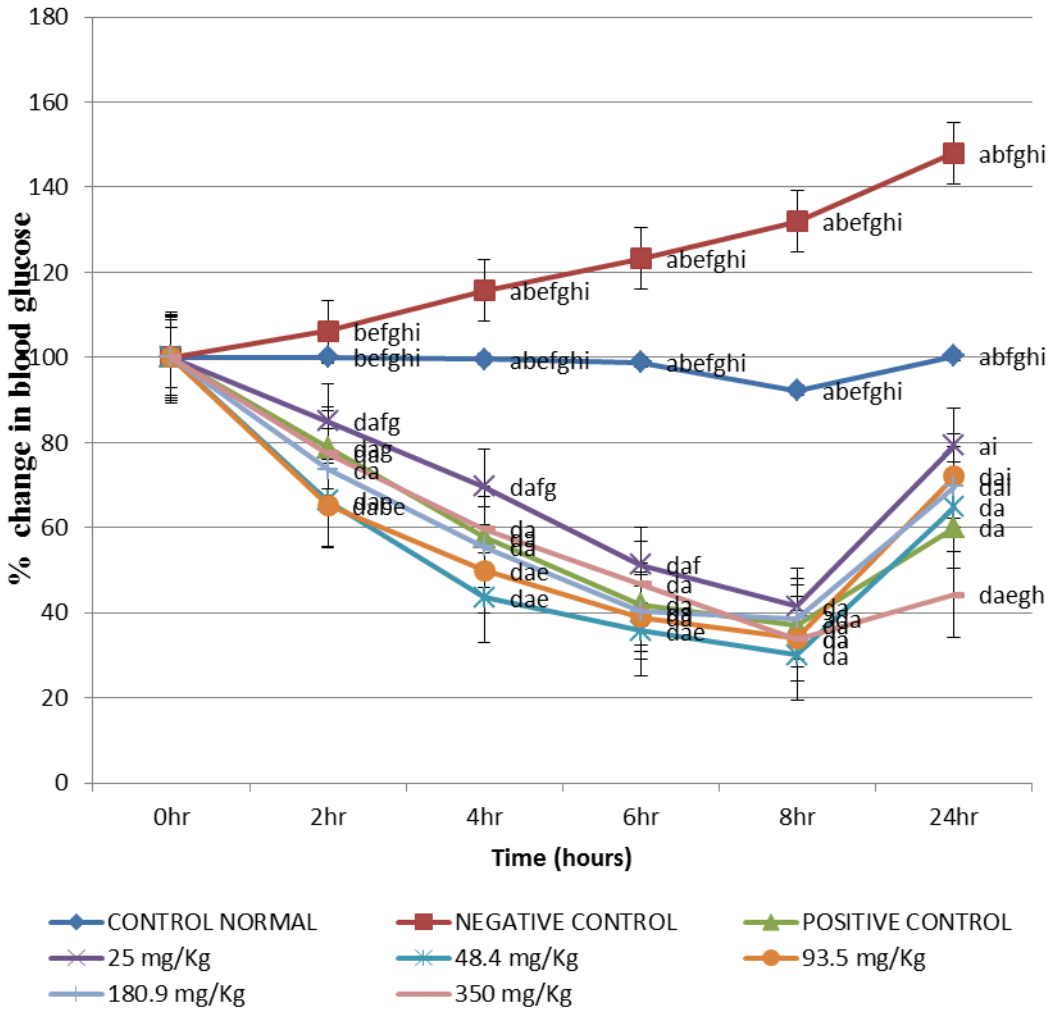

Figure 4.1: Percentage reduction in blood glucose levels at varying times after oral administration of the aqueous extracts of *Berberis holstii* in alloxan induced diabetic male mice.

Values are expressed as % means ± SEM for five animals for each time point. $^{d}\rho < 0.05$ when compared to normal control; $^{a}\rho < 0.05$ when compared to negative control; $^{b}\rho < 0.05$ when compared to positive control; $^{e}\rho < 0.05$ when compared to 25 mg/kg body weight; $^{f}\rho < 0.05$ when compared to 48.4 mg/kg body weight; $^{g}\rho < 0.05$ when compared to 93.5 mg/kg body weight; $^{h}\rho < 0.05$ when compared to 180 mg/kg body weight; $^{i}\rho < 0.05$ when compared to 350 mg/kg body weight.

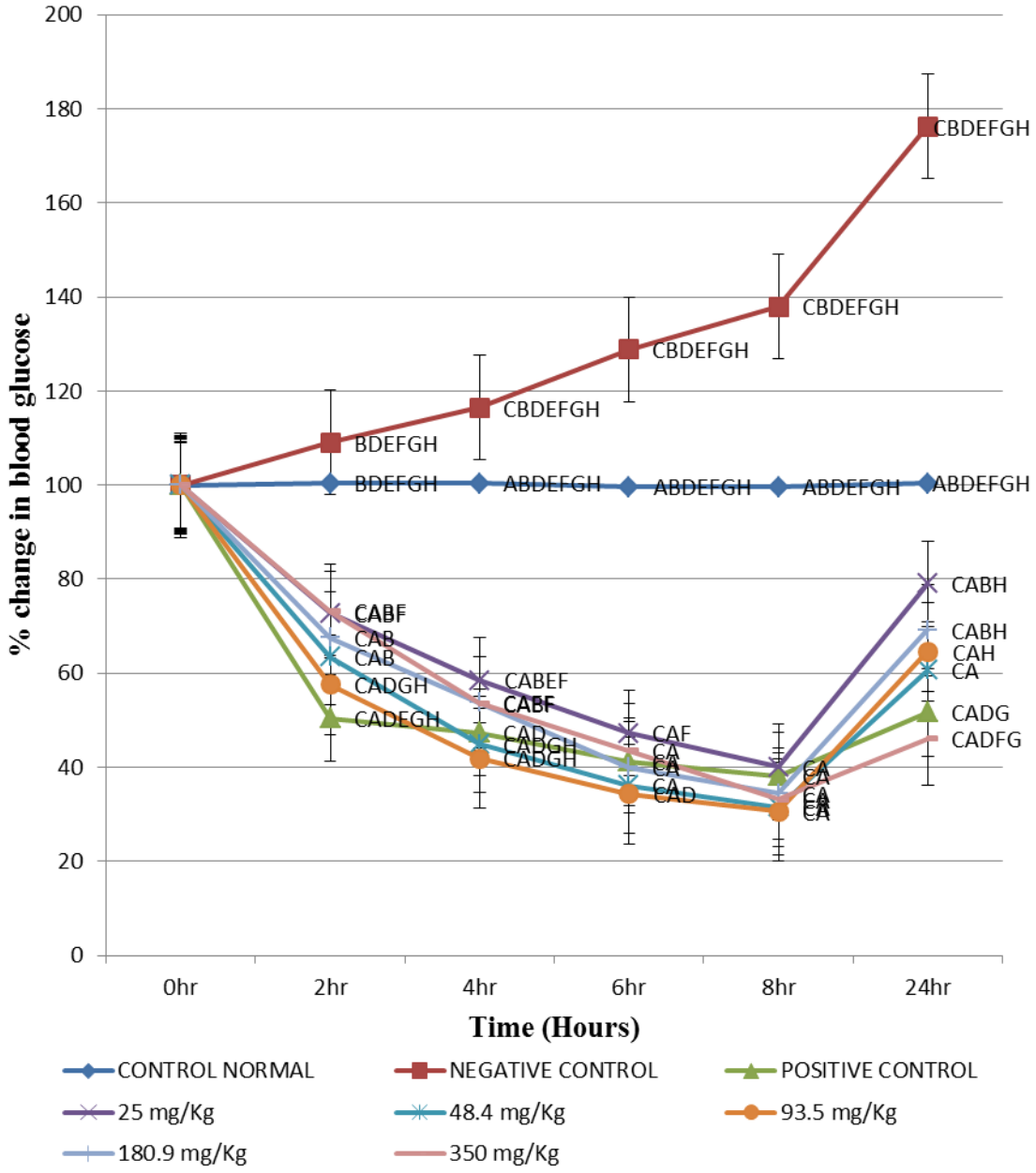

Figure 4.2: Percentage reduction in blood glucose levels at varying times after intraperitoneal administration of the aqueous extracts of *Berberis holstii* in alloxan induced diabetic male mice.

Values are expressed as % means ± SEM for five animals for each time point. $^{C}\rho < 0.05$ when compared to normal control; $^{A}\rho < 0.05$ when compared to negative control; $^{B}\rho < 0.05$ when compared to positive control; $^{D}\rho < 0.05$ when compared to 25 mg/kg body weight; $^{E}\rho < 0.05$ when compared to 48.4 mg/kg body weight; $^{F}\rho < 0.05$ when compared to 93.5 mg/kg body weight; $^{G}\rho < 0.05$ when compared to 180 mg/kg body weight; $^{H}\rho < 0.05$ when compared to 350 mg/kg body weight.

Table 4.3 shows the pharmacokinetics of the hypoglycemic activity for the first four hours of the aqueous leaf extracts of *B. holstii* for the five doses orally and intraperitoneally. Results indicate that the pseudo-first order rate constants for the orally administered doses of the aqueous leaf extracts of *B. holstii* at 25 and 48.4 mg/kg was 0.1813 and 0.4152 and their accompanying half-live's were 3.82 and 1.67 while those of the other three doses were similar. The half-life of the 25 mg/kg dose was higher than that of glibenclamide while those of the other doses were lower. Pseudo-first order rate constants for the intraperitoneally administered doses of aqueous leaf extracts of *B. holstii* at 25, 48.4 and 93.5 mg/kg were 0.268, 0.4006 and 0.4348, respectively, and their accompanying half-lives were 2.59, 1.73 and 1.59, respectively, while those of the other two doses were similar. The half-lives of the intraperitoneally administered doses of the aqueous leaf extracts of *B. holstii* at 25, 180.9 and 350 mg/kg were higher relative to those of insulin while those of the other doses were lower.

The rate constants for the orally administered aqueous extracts for the five doses ranged from 0.1813 to 0.4152 per hour and the half-lifes ranged from 3.82 to 1.67 hours, respectively. Rate constants for the intraperitoneally administered aqueous extracts for the five doses ranged from 0.268 to 0.4348 per hour and the half-lifes ranged from 2.59 to 1.73 hours, respectively. The rate constant for insulin was 0.3732 per hour and that of glibenclamide was 0.3106 per hour while their corresponding half-lifes were 1.86 and 2.23 hours, respectively.

Table 4.3: Pharmacokinetics of the hypoglycemic activity for the first four hours of the five doses of the aqueous extracts of *B. holstii*

Drug (dose)	Route	Rate constant ($hour^{-1}$)	Half-life (hours)
Insulin	IP	0.3732	1.86
Glibenclamide	Oral	0.3106	2.23
Extract (mg/kg bw)			
25	IP	0.2680	2.59
	Oral	0.1813	3.82
48.4	IP	0.4006	1.73
	Oral	0.4152	1.67
93.4	IP	0.4348	1.59
	Oral	0.3680	1.88
180.9	IP	0.3107	2.23
	Oral	0.3680	1.88
350	IP	0.3123	2.22
	Oral	0.3680	1.88

Results are expressed as Means of five mice for each time point; bw represents body weight

4.1.2 Effects of administration of *Piper capense* extracts on blood glucose levels in alloxan induced diabetic mice

Piper capense root yielded a red brown lyophilate whose concentration was 100 mg/g dry weight. Oral administration of aqueous extracts of *Piper capense* at 25, 48.4, 93.5, 180.9, and 350 mg/kg body weight to diabetic mice decreased the blood sugar levels significantly as from the 2nd hour independent of the dose. During the 2nd hour, the percentage reduction of blood glucose levels in mice by the aqueous extracts of *Piper capense* at 25, 48.4, 93.5, 180.9 and 350 mg/kg body weight were 47.8, 57.2, 58.7, 57.5 and 47.4%, respectively, compared to glibenclamide-treated diabetic mice whose blood sugar levels was lowered to 71.7% in the same hour (Figure 4.3). At this hour, the plant extract lowered the blood glucose significantly to levels higher than the normal control mice ($^{d}\rho < 0.05$) (Table 4.4). However, the dose significantly lowered blood glucose levels in comparison to diabetic control mice ($^{a}\rho < 0.05$) (Table 4.4).

During the 4th hour, the glucose lowering effect by the five dose levels of *Piper capense* was also observed; the percentage reduction of blood glucose was 36.2, 43.4, 40.7, 44.1 and 30.1%, respectively, compared to glibenclamide treated diabetic mice whose blood glucose levels was lowered to 51.1% within that hour. In this hour, the five aqueous extracts of *Piper capense* lowered blood glucose levels in diabetic mice to normal levels ($^{d}\rho > 0.05$) and even to levels lower than those caused by glibenclamide ($^{b}\rho < 0.05$). By the 6th hour, the five aqueous extracts doses of *Piper capense* lowered blood sugar levels in diabetic mice to 32.5, 33.3, 32.9, 37.9 and 25.7%, respectively, compared to glibenclamide treated diabetic mice whose blood sugar levels was lowered to 40.9%. At this hour, the five aqueous extracts of *Piper capense* at 180.9 mg/kg body weight lowered blood glucose levels in diabetic mice as effectively as glibenclamide ($^{b}\rho < 0.05$). During the 8th hour, the same trend in blood glucose by the five aqueous extracts of *Piper capense* lowered blood glucose in diabetic mice to levels lower than glibenclamide, which lowered it to 33.2%. During this hour, the blood glucose lowering effect by the five aqueous extracts of *Piper capense* reduced blood glucose levels in diabetic mice to 30.0, 27.3, 27.5, 32.9 and 21.7%, respectively, relative to that of the diabetic control mice (Figure 4.3). At the 24th hour, the blood glucose levels in diabetic mice treated with the five extracts doses of *Piper capense* in diabetic mice had returned to the diabetic state.

Intraperitoneal administration of aqueous extracts of *Piper capense* at 25, 48.4, 93.5, 180.9, and 350 mg/kg body weight to mice decreased the blood sugar levels in diabetic mice significantly as from the 2nd hour independent of the dose compared to

control. During the 2nd hour, the percentage reduction of blood glucose levels in diabetic mice by the aqueous extract of *Piper capense* at 25, 48.4, 93.5, 180.9, and 350 mg/kg body weight was 70.4, 55.7, 55.5, 51.7 and 48.6%, respectively, compared to insulin-treated diabetic mice whose blood sugar levels was lowered to 43.89% within the same hour (Figure 4.4). However, the five aqueous extracts doses of *Piper capense* significantly lowered blood glucose levels in diabetic mice in relative to that of the diabetic control mice ($^{\mathbf{A}}\rho < 0.05$) (Table 4.4).

In the 4th hour, the glucose lowering effect by the five dose levels was also observed; the percentage reduction of blood glucose in diabetic mice was 54,0, 42.7, 37.9, 36.4 and 31.8%, respectively, compared to insulin treated diabetic mice whose blood sugar levels was lowered to 41.3%. The five aqueous extracts of *Piper capense* lowered blood glucose levels in diabetic mice to normal in this hour ($^{\mathbf{C}}\rho > 0.05$). By the 6th hour, the five extract doses of *Piper capense* lowered blood sugar levels in diabetic mice to 44.9, 34.8, 30.5, 29.5 and 25.1%, respectively, compared to insulin treated diabetic mice whose blood sugar levels was reduced to 37.64%. At this hour, the aqueous extract of *Piper capense* at 48.4 mg/kg body weight lowered blood glucose levels in diabetic mice as effectively as insulin ($^{\mathbf{B}}\rho < 0.05$). During the 8th hour, the same trend in blood glucose reduction by the five aqueous extracts of *Piper capense* lowered blood glucose in diabetic mice to levels lower than insulin, which lowered it to 35.4%. In this hour, the five aqueous extracts of *Piper capense* lowered blood glucose in diabetic mice to 37.5, 28.1, 23.3, 24.6 and 18.6%, respectively (Figure 4.4).

All the diabetic mice treated with the five aqueous extracts doses of *Piper capense* in diabetic mice had returned to the diabetic state by the 24^{th} hour.

Oral and intraperitoneal administration of aqueous extracts of *Piper capense* at 25, 48.4, 93.5, 180.9 and 350 mg/kg body weight doses lowered blood glucose in diabetic mice to similar levels ($\rho > 0.05$; Table 4.4).

Table 4.4 Effects of administration of five therapeutic doses of aqueous extracts of *Piper capense* at different times on blood glucose levels in alloxan induced diabetic mice

Treatment	Route	Levels of Glucose at Varying Times (mmol/L)					
		0 hour	2 hour	4 hour	6 hour	8 hour	24 hour
Normal control	IP	5.26 ± 0.05^{ABDEFGH}	$5.32\pm0.06^{\mathbf{ADE}}$	$5.36\pm0.05^{\mathbf{A}}$	$5.22\pm0.37^{\mathbf{A}}$	$5.30\pm0.04^{\mathbf{AH}}$	$5.32\pm0.05^{\mathbf{ADEF}}$
	Oral	5.18 ± 0.04^{abefghi}	$5.18\pm0.04^{\mathbf{abg}}$	$5.20\pm0.03^{\mathbf{ab}}$	$5.16\pm0.02^{\mathbf{ai}}$	$5.20\pm0.03^{\mathbf{afi}}$	$5.22\pm0.04^{\mathbf{aef}}$
Negative control	IP	$14.70\pm1.08^{\mathbf{C}}$	15.86 ± 1.13^{CBDEFGH}	17.14 ± 1.27^{CBDEFGH}	18.58 ± 1.34^{CBDEFGH}	20.18 ± 1.32^{CBDEFGH}	23.60 ± 1.54^{CBDEFGH}
	Oral	$13.42\pm0.95^{\mathbf{d}}$	$14.76\pm0.76^{\mathbf{bdefghi}}$	16.20 ± 0.65^{dbefghi}	17.34 ± 0.61^{dbefghi}	19.26 ± 0.62^{dbefghi}	21.66 ± 1.14^{dbefghi}
Positive control (insulin)	IP	$14.40\pm0.67^{\mathbf{C}}$	$6.32\pm0.27^{\mathbf{AD}}$	$5.94\pm0.20^{\mathbf{A}}$	$5.42\pm0.16^{\mathbf{A}}$	$5.10\pm0.11^{\mathbf{AH}}$	$6.82\pm0.20^{\mathbf{AD}}$
Positive control (glibenclamide)	Oral	$15.60\pm1.33^{\mathbf{d}}$	$11.18\pm1.67^{\mathbf{defi}}$	$8.10\pm0.87^{\mathbf{daefgi}}$	$6.38\pm0.41^{\mathbf{afgi}}$	$5.18\pm0.12^{\mathbf{afi}}$	$8.18\pm0.83^{\mathbf{a}}$
Extract dose (mg/kg body weight)							
25	IP	$14.30\pm0.69^{\mathbf{C}}$	$10.06\pm0.65^{\mathbf{CAB}}$	$7.72\pm0.56^{\mathbf{AH}}$	$6.42\pm0.35^{\mathbf{AH}}$	$5.36\pm0.25^{\mathbf{AH}}$	$10.70\pm0.55^{\mathbf{CABH}}$
	Oral	$13.86\pm0.28^{\mathbf{d}}$	$6.62\pm0.78^{\mathbf{ab}}$	$5.02\pm0.36^{\mathbf{ab}}$	$4.50\pm0.36^{\mathbf{a}}$	$4.16\pm0.35^{\mathbf{a}}$	$9.82\pm0.44^{\mathbf{da}}$
48.4	IP	$14.58\pm0.67^{\mathbf{C}}$	$8.12\pm0.45^{\mathbf{CA}}$	$6.22\pm0.46^{\mathbf{A}}$	$5.08\pm0.32^{\mathbf{A}}$	$4.10\pm0.45^{\mathbf{A}}$	$9.62\pm0.47^{\mathbf{CAH}}$
	Oral	$11.88\pm1.85^{\mathbf{d}}$	$6.80\pm0.74^{\mathbf{ab}}$	$5.16\pm0.62^{\mathbf{ab}}$	$3.96\pm0.45^{\mathbf{ab}}$	$3.24\pm0.31^{\mathbf{dab}}$	$9.40\pm0.43^{\mathbf{da}}$
93.5	IP	$14.42\pm0.47^{\mathbf{C}}$	$8.00\pm0.14^{\mathbf{A}}$	$5.46\pm0.43^{\mathbf{A}}$	$4.40\pm0.40^{\mathbf{A}}$	$3.36\pm0.20^{\mathbf{A}}$	$8.68\pm0.56^{\mathbf{CA}}$
	Oral	$13.38\pm0.37^{\mathbf{d}}$	$7.86\pm0.50^{\mathbf{a}}$	$5.44\pm0.53^{\mathbf{ab}}$	$4.40\pm0.35^{\mathbf{ab}}$	$3.68\pm0.34^{\mathbf{a}}$	$8.38\pm0.74^{\mathbf{a}}$
180.9	IP	$14.16\pm0.61^{\mathbf{C}}$	$7.32\pm0.51^{\mathbf{A}}$	$5.16\pm0.34^{\mathbf{A}}$	$4.18\pm0.31^{\mathbf{A}}$	$3.48\pm0.21^{\mathbf{A}}$	$8.54\pm0.63^{\mathbf{A}}$
	Oral	$12.60\pm0.72^{\mathbf{d}}$	$7.24\pm0.67^{\mathbf{a}}$	$5.56\pm0.60^{\mathbf{a}}$	$4.78\pm0.58^{\mathbf{a}}$	$4.14\pm0.87^{\mathbf{a}}$	$8.40\pm0.81^{\mathbf{a}}$
350	IP	$13.78\pm0.74^{\mathbf{C}}$	$6.70\pm0.53^{\mathbf{AD}}$	$4.38\pm0.20^{\mathbf{AD}}$	$3.46\pm0.29^{\mathbf{AD}}$	$2.56\pm0.14^{\mathbf{ABD}}$	$5.54\pm0.38^{\mathbf{ADE}}$
	Oral	$12.28\pm0.55^{\mathbf{d}}$	$5.82\pm1.08^{\mathbf{ab}}$	$3.70\pm0.23^{\mathbf{ab}}$	$3.16\pm0.28^{\mathbf{dab}}$	$2.66\pm0.26^{\mathbf{dab}}$	$7.10\pm0.57^{\mathbf{a}}$

Results are expressed as Means ± Standard Deviation for five mice per group. Means accompanied by similar upper case letters and similar lower case letters in the same column are significantly different at $\rho \leq 0.05$ by ANOVA and post ANOVA (Bonferroni-Holm) test. Means for IP administration: $^{C}\rho < 0.05$ with respect to normal control; $^{A}\rho < 0.05$ with respect to negative control; $^{\mathbf{B}}\rho < 0.05$ with respect to positive control; $^{\mathbf{D}}\rho < 0.05$ with respect to 25 mg/kg body weight; $^{\mathbf{E}}\rho < 0.05$ with respect to 48.4 mg/kg body weight; $^{\mathbf{F}}\rho < 0.05$ with respect to 93.5 mg/kg body weight; $^{\mathbf{G}}\rho < 0.05$ with respect to 180 mg/kg body weight; $^{\mathbf{H}}\rho < 0.05$ with respect to 350 mg/kg body weight. Means for Oral administration: $^{\mathbf{d}}\rho < 0.05$ with respect to normal control; $^{a}\rho < 0.05$ with respect to negative control; $^{\mathbf{b}}\rho < 0.05$ with respect to positive control $^{\mathbf{e}}\rho < 0.05$ with respect to 25 mg/kg body weight; $^{\mathbf{f}}\rho < 0.05$ with respect to 48.4 mg/kg body weight; $^{\mathbf{g}}\rho < 0.05$ with respect to 93.5 mg/kg body weight; $^{\mathbf{h}}\rho < 0.05$ with respect to 180 mg/kg body weight; $^{\mathbf{i}}\rho < 0.05$ with respect to 350 mg/kg body weight. Value followed by $*\rho < 0.05$ is considered statistically significant when the mean of the oral group is compared to intraperitoneal group by T-test.

Table 4.5: Effects of administration of five therapeutic doses of aqueous extracts of *Piper capense* at different times on blood glucose levels in alloxan induced diabetic mice

Treatment	Route	Levels of Glucose at Varying Times (mmol/L)					
		0 hour	2 hour	4 hour	6 hour	8 hour	24 hour
Normal control	IP	5.26±0.05	5.32±0.06	5.36±0.05	5.22±0.37	5.30±0.04	5.32±0.05
	Oral	5.18±0.04	5.18±0.04	5.20±0.03	5.16±0.02	5.20±0.03	5.22±0.04
Negative control	IP	14.70±1.08^{kmnop}	15.86±1.13^{kmnop}	17.14±1.27^{kmnop}	18.58±1.34^{kmnopq}	20.18±1.32^{kmnopq}	23.60±1.54^{opq}
	Oral	13.42±0.95^{JLN}	14.76±0.76^{JLNQ}	16.20±0.65^{JLNQR}	17.34±0.61^{LNQRS}	19.26±0.62^{NQR}	21.66±1.14^{RS}
Positive control (insulin)	IP	14.40±0.67	6.32±0.27^{mnopq}	5.94±0.20^{mnopq}	5.42±0.16^{mnopq}	5.10±0.11^{mnop}	6.82±0.20^{mnoq}
Positive control (glibenclamide)	Oral	15.60±1.33^{JLR}	11.18±1.67^{JLNS}	8.10±0.87^{LNQS}	6.38±0.41^{NQRS}	5.18±0.12^{NQRS}	8.18±0.83^{LNQRS}
Extract dose (mg/kg body weight)							
25	IP	14.30±0.69	10.06±0.65^{mnq}	7.72±0.56^{mnop}	6.42±0.35^{nop}	5.36±0.25^{nop}	10.70±0.55^{mq}
	Oral	13.86±0.28	6.62±0.78^{LN}	5.02±0.36^{LNQR}	4.50±0.36^{LNQR}	4.16±0.35^{NQR}	9.82±0.44
48.4	IP	14.58±0.67	8.12±0.45^{mnq}	6.22±0.46^{mnop}	5.08±0.32^{nop}	4.10±0.45^{nop}	9.62±0.47^{mq}
	Oral	11.88±1.85^{JS}	6.80±0.74^{LNQRS}	5.16±0.62^{LNQR}	3.96±0.45^{LNQR}	3.24±0.31^{LNR}	9.40±0.43^{JLQS}
93.5	IP	14.42±0.47	8.00±0.14^{mq}	5.46±0.43^{no}	4.40±0.40^{nop}	3.36±0.20^{op}	8.68±0.56^{mq}
	Oral	13.38±0.37	7.86±0.50^{LS}	5.44±0.53^{NQR}	4.40±0.35^{NQR}	3.68±0.34^{NQR}	8.38±0.74^{LS}
180.9	IP	14.16±0.61	7.32±0.51^{mnq}	5.16±0.34^{mnop}	4.18±0.31^{nop}	3.48±0.21^{nop}	8.54±0.63^{mq}
	Oral	12.60±0.72	7.24±0.67^{LNQS}	5.56±0.60^{LNQRS}	4.78±0.58^{LNQR}	4.14±0.87^{NQR}	8.40±0.81^{LNS}
350	IP	13.78±0.74	6.70±0.53^{mq}	4.38±0.20^{nopq}	3.46±0.29^{nop}	2.56±0.14^{nop}	5.54±0.38^{mnq}
	Oral	12.28±0.55	5.82±1.08^{LNS}	3.70±0.23^{LNQR}	3.16±0.28^{NQR}	2.66±0.26^{NQR}	7.10±0.57^{LS}

Results are expressed as Means ± Standard Deviation for five mice per group. Means accompanied by similar upper case letters and similar lower case letters in the same row are not significantly different at $\rho \leq 0.05$ by ANOVA and post ANOVA (Bonferroni-Holm) test.

Means for IP administration: $^{k}\rho < 0.05$ with respect to 0 hr; $^{m}\rho < 0.05$ with respect to 2 hr; $^{n}\rho < 0.05$ with respect to 4 hr; $^{o}\rho < 0.05$ with respect to 6 hr; $^{p}\rho < 0.05$ with respect to 8 hr; $^{q}\rho < 0.05$ with respect to 24 hr. Means for Oral administration: $^{J}\rho < 0.05$ with respect to 0 hr; $^{L}\rho < 0.05$ with respect to 2 hr; $^{N}\rho < 0.05$ with respect to 4 hr; $^{Q}\rho < 0.05$ with respect to 6 hr; $^{R}\rho < 0.05$ with respect to 8 hr; $^{S}\rho < 0.05$ with respect to 24 hr.

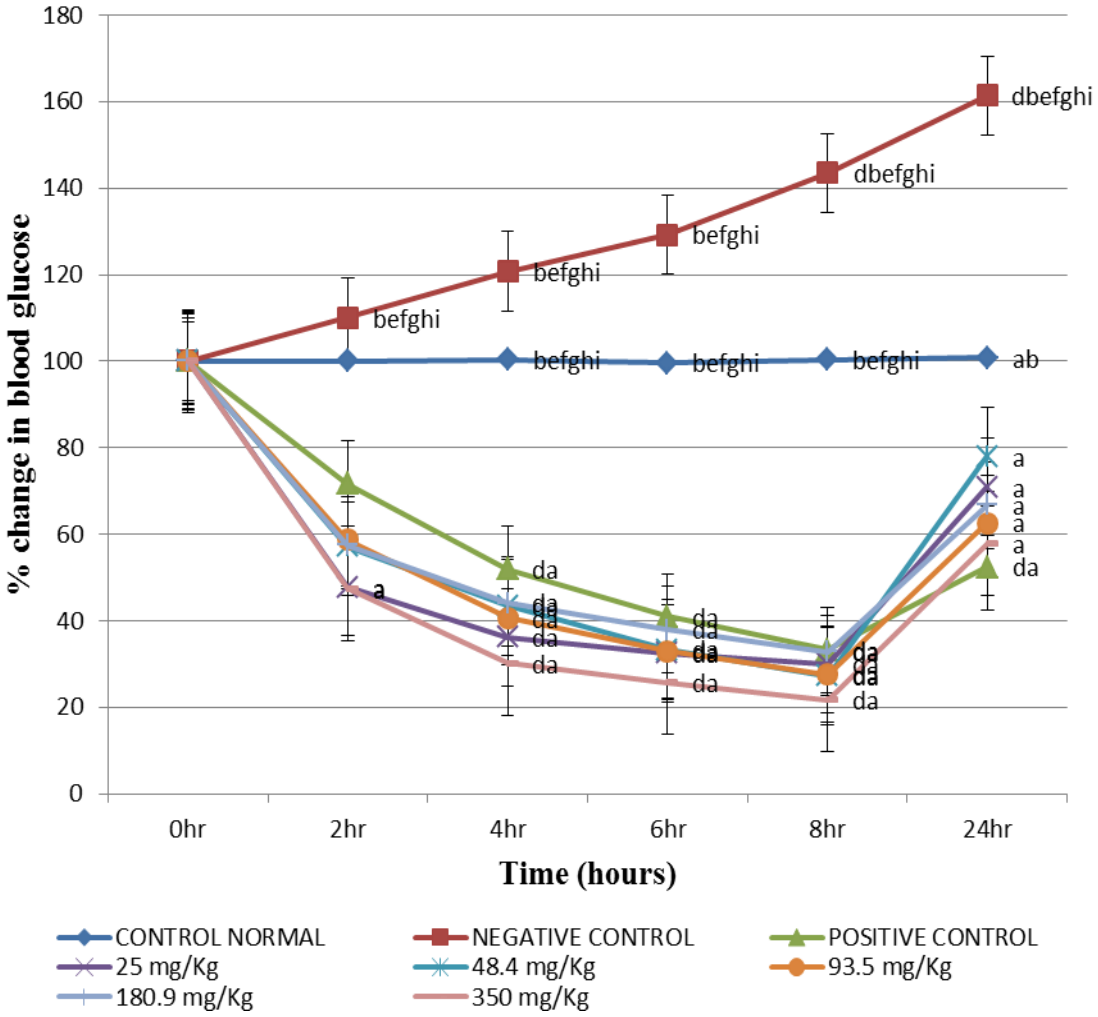

Figure 4.3: Percentage reduction in blood glucose levels at varying times after oral administration of the aqueous extracts of *Piper capense* in alloxan induced diabetic mice.

Values are expressed as % means ± SEM for five animals for each time point. $^{d}\rho < 0.05$ when compared to normal control; $^{a}\rho < 0.05$ when compared to negative control; $^{b}\rho < 0.05$ when compared to positive control; $^{e}\rho < 0.05$ when compared to 25 mg/kg body weight; $^{f}\rho < 0.05$ when compared to 48.4 mg/kg body weight; $^{g}\rho < 0.05$ when compared to 93.5 mg/kg body weight; $^{h}\rho < 0.05$ when compared to 180 mg/kg body weight; $^{i}\rho < 0.05$ when compared to 350 mg/kg body weight.

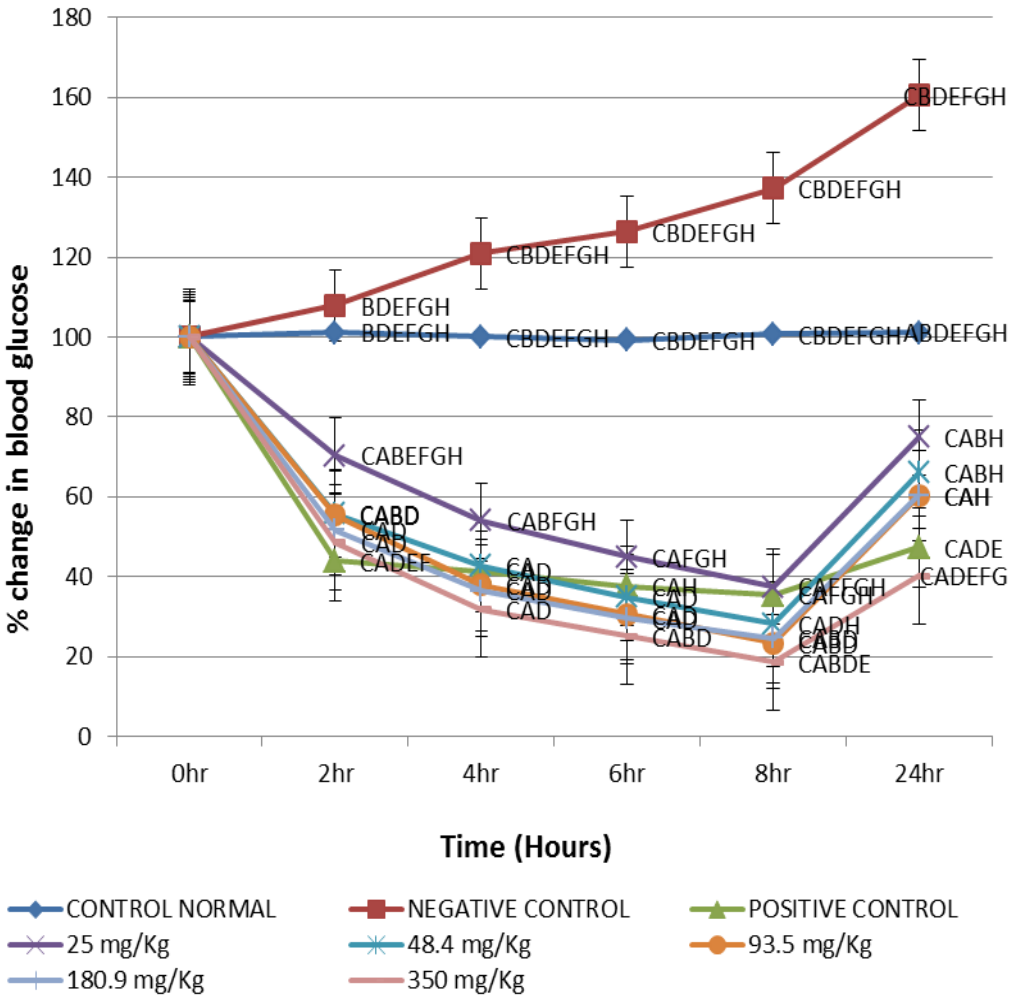

Figure 4.4: Percentage reduction in blood glucose levels at varying times after intraperitoneal administration of the aqueous extracts of *Piper capense* in alloxan induced diabetic male mice.
Values are expressed as % means ± SEM for five animals for each time point. $^{C}\rho < 0.05$ when compared to normal control; $^{A}\rho < 0.05$ when compared to negative control; $^{B}\rho < 0.05$ when compared to positive control; $^{D}\rho < 0.05$ when compared to 25 mg/kg body weight; $^{E}\rho < 0.05$ when compared to 48.4 mg/kg body weight; $^{F}\rho < 0.05$ when compared to 93.5 mg/kg body weight; $^{G}\rho < 0.05$ when compared to 180 mg/kg body weight; $^{H}\rho < 0.05$ when compared to 350 mg/kg body weight.

Table 4.6 shows the pharmacokinetics of the hypoglycemic activity for the first four hours of the aqueous leaf extracts of *P. capense* for the five doses. Results indicate that the pseudo-first order rate constants for the orally administered doses of the aqueous leaf extracts of *P. capense* at 25, 48.4, 93.5, 180.9 and 350 mg/kg were 0.5078, 0.417, 0.45, 0.409 and 0.5998, respectively, and their accompanying

half-lives were 1.36, 1.66, 1.54, 1.69, and 1.16, respectively. These half-lives were lower than that of glibenclamide. Pseudo-first order rate constants for the intraperitoneally administered doses of aqueous leaf extracts of *P. capense* at 25, 48.4, 93.5, 180.9 and 350 mg/kg were 0.3082, 0.417, 0.4856, 0.4783 and 0.5731, respectively, and their accompanying half-lives were 2.25, 1.66, 1.43, 1.45 and 1.21, respectively. The half-lifes of 25, and 48.4 mg/kg doses were higher than those of insulin while those of the other doses were lower. The rate constants for the orally administered aqueous extracts of *P. capense* for the five doses ranged from 0.409 to 0.5998 per hour and the half-lifes ranged from 1.69 to 1.69 hours, respectively. Rate constants for the intraperitoneally administered aqueous extracts of *P. capense* for the five doses ranged from 0.3082 to 0.5731 per hour and the half-lifes ranged from 2.25 to 1.21 hours, respectively. The rate constant for insulin was 0.4428 per hour and that of glibenclamide was 0.3277 per hour while their corresponding half-lifes were 1.57 and 2.11 hours, respectively.

Table 4.6 Pharmacokinetics of the hypoglycemic activity for the first four hours of the five doses of the aqueous extracts of *P. capense*

Drug (dose)	Route	Rate constant ($hour^{-1}$)	Half-life (hours)
Insulin	IP	0.4428	1.57
Glibenclamide	Oral	0.3277	2.11
Extract (mg/kg bw)			
25	IP	0.3082	2.25
	Oral	0.5078	1.36
48.4	IP	0.4170	1.66
	Oral	0.4170	1.66
93.4	IP	0.4856	1.43
	Oral	0.4500	1.54
180.9	IP	0.4783	1.45
	Oral	0.4090	1.69
350	IP	0.5731	1.21
	Oral	0.5998	1.16

Results are expressed as Means of five mice for each time point; bw represents body weight

4.1.3 Effects of administration of *Vernonia lasiopus* extracts on blood glucose levels in male diabetic mice

The aqueous leaves extract of *Vernonia lasiopus* produced a brown lyophilate whose concentration was 90 mg/g dry weight. Oral administration of aqueous extracts of *Vernonia lasiopus* at 25, 48.4, 93.5, 180.9, and 350 mg/kg body weight to mice decreased the blood sugar levels significantly as from the 2nd hour independent of the dose. During the 2nd hour, the percentage reduction of blood glucose levels in mice by the aqueous extract of *Vernonia lasiopus* at 25, 48.4, 93.5, 180.9, and 350 mg/kg body weight were 74.7, 62.9, 67.2, 60.0 and 69.7%, respectively, compared to glibenclamide treated diabetic mice whose blood sugar levels was lowered to 66.5% (Figure 4.5). At this hour, the five aqueous extract doses significantly lowered the blood glucose levels but not to normal levels ($^{\mathbf{d}}\rho < 0.05$). The five extract doses, however, significantly lowered blood glucose levels in diabetic mice relative to the diabetic control ($^{\mathbf{a}}\rho < 0.05$) (Table 4.7).

In the 4th hour, the glucose lowering effect by the five dose levels was also observed; the percentage reduction of blood glucose was 56.5, 45.5, 46.7, 48.1 and 51.2%, respectively, compared to glibenclamide-treated diabetic mice whose blood sugar levels was lowered to 49% within that hour. By the 6th hour, the five extract doses lowered blood sugar levels to 48.6, 32.6, 31.0, 38.7 and 37.8%, respectively, compared to glibenclamide treated diabetic mice whose blood sugar levels was lowered to 38.3%. The blood glucose level in diabetic mice was lowered to normal levels by the five extract doses ($^{\mathbf{d}}\rho > 0.05$). At this hour, the extract dose of 180.9 mg/kg body weight lowered blood glucose levels as effectively as glibenclamide ($^{\mathbf{b}}\rho < 0.05$). During the 8th hour, the same trend in blood glucose lowering by administration of the five aqueous extracts doses of *Vernonia lasiopus* in diabetic

mice lowered blood glucose levels to 42.8, 28.0, 25.4, 30.9 and 29.6%, respectively, which was lower than that of glibenclamide (31.7%) (Figure 4.5). At the 24th hour, the blood glucose levels in the diabetic mice administered with 180.9 mg/kg body weight doses had not returned to the diabetic state but those administered with 25, 48.4 and 93.5 mg/kg body weight doses had returned to the diabetic state (Table 4.7).

Intraperitoneal administration of aqueous extracts of *Vernonia lasiopus* at 25, 48.4, 93.5, 180.9, and 350 mg/kg body weight to mice decreased the blood sugar levels significantly as from the 2nd hour independent of the dose. During the 2nd hour, the percentage reduction of blood glucose levels in diabetic mice by the aqueous extract of *Vernonia lasiopus* at 25, 48.4, 93.5, 180.9, and 350 mg/kg body weight was 51.9, 39.3, 44.1, 49.2 and 52.5%, respectively, compared to insulin-treated diabetic mice whose blood sugar levels was lowered to 40.9% (Figure 4.6). The five aqueous extract doses, however, significantly lowered blood glucose levels in diabetic mice relative to the diabetic control mice ($^{\mathbf{A}}\rho < 0.05$) (Table 4.7).

In the 4th hour, the glucose lowering effect by the five dose levels was also observed; the percentage reduction of blood glucose was 41.1, 31.9, 37.3, 36.0 and 36.8%, respectively, compared to insulin-treated diabetic mice whose blood sugar levels was lowered to 37.1%. The blood glucose level in diabetic mice was lowered to normal by the five extracts doses ($^{\mathbf{C}}\rho > 0.05$). By the 6th hour, the five extract doses lowered blood sugar levels to 32.3, 23.6, 30.5, 26.1 and 37.5%, respectively, compared to insulin treated diabetic mice whose blood sugar levels was lowered to 35.6%. At this hour, the extract dose of 93.5 mg/kg body weight

dose lowered blood glucose levels in diabetic mice as effectively as insulin ($^{B}\rho < 0.05$). During the 8th hour, the same trend in blood glucose lowering effect was observed where the five aqueous extracts doses in diabetic mice lowered blood glucose to levels lower than that of insulin (33%). Here, the blood glucose levels in diabetic mice were reduced by the five extracts doses of *Vernonia lasiopus* to 29.8, 20.9, 26.4, 21.9 and 23.7%, respectively (Figure 4.6). In the 24th hour, diabetic mice intraperitoneally administered with aqueous extracts of *Vernonia lasiopus* at 25 and 48.4 mg/kg body weight doses had returned to the diabetic state but those administered with aqueous extracts of *Vernonia lasiopus* at 93.5, 180.9 and 350 mg/kg body weight doses had not (Table 4.8a). Oral and intraperitoneal administration of aqueous extracts of *Vernonia lasiopus* at all the five doses to diabetic mice lowered blood glucose to similar levels (Table 4.7).

Table 4.7 Effects of administration of five therapeutic doses of aqueous extracts of *Vernonia lasiopus* at different times on blood glucose levels in alloxan induced diabetic mice

Treatment	Route	Levels of Glucose at Varying Times (mmol/L)					
		0 hour	2 hour	4 hour	6 hour	8 hour	24 hour
Normal control	IP	5.18±0.20^{ABDEFGH}	5.20±0.03^{A}	5.16±0.02^{A}	5.18±0.04^{A}	5.16±0.02^{AG}	5.24±0.02^{ADE}
	Oral	5.18±0.02^{abefghi}	5.20±0.03^{abefghi}	5.16±0.02^{abef}	5.18±0.04^{a}	5.16±0.02^{a}	5.24±0.02^{abefg}
Negative control	IP	13.90±1.28^{C}	15.10±1.29^{CBDEFGH}	16.42±1.10^{CBDEFGH}	17.84±1.16^{CBDEFGH}	19.06±1.13^{CBDEFGH}	22.62±1.06^{CBDEFGH}
	Oral	13.42±1.32^{befghid}	15.12±1.20^{dbefghi}	17.42±1.11^{dbefghi}	18.84±1.18^{dbefghi}	19.08±1.14^{dbefghi}	22.80±1.12^{dbefghi}
Positive control (insulin)	IP	15.78±1.26^{C}	6.46±0.29^{CA}	5.86±0.24^{A}	5.62±0.20^{A}	5.20±0.14^{AEG}	6.90±0.16^{AD}
Positive control (glibenclamide)	Oral	16.42±1.38^{aefghi}	10.92±0.96^{efghi}	8.04±0.45^{efghi}	6.28±0.34^{defghi}	5.20±0.11^{defghi}	8.18±0.53^{efgh}
Extract dose (mg/kg body weight)							
25	IP	13.88±0.48^{C}	7.20±0.55^{A}	5.70±0.24^{A}	4.48±0.13^{A}	4.14±0.09^{A}	9.84±0.38^{CBFGH}
	Oral	13.98±1.29^{d}	10.44±0.82^{da}	7.90±0.55^{da}	6.80±0.44^{a}	5.98±0.48^{ag}	9.78±0.66^{dahi}
48.4	IP	14.24±0.84^{C}	5.60±0.48^{A}	4.54±0.46^{A}	3.36±0.26^{A}	2.98±0.22^{A}	8.02±0.12^{CADH}
	Oral	14.44±0.96^{d}	9.08±0.43^{da}	6.50±0.39^{a}	4.70±0.21^{a}	4.04±0.26^{a}	8.20±0.40^{dai}
93.5	IP	14.10±0.23^{C}	6.22±0.28^{A}	5.26±0.27^{A}	4.30±0.32^{A}	3.72±0.18^{A}	7.30±0.29^{AD}
	Oral	19.44±0.64^{d}	9.70±0.58^{da}	6.74±0.72^{a}	4.48±0.37^{ae}	3.66±0.21^{ae}	7.90±0.36^{dai}
180.9	IP	13.16±0.67^{C}	6.48±0.70^{A}	4.74±0.62^{A}	3.44±0.29^{A}	2.88±0.32^{CAB}	6.94±0.20^{AD}
	Oral	14.78±0.83^{d}	8.86±0.45^{da}	7.12±0.36^{a}	5.72±0.42^{a}	4.56±0.45^{a}	7.16±0.22^{ae}
350	IP	12.66±0.71^{C}	6.64±0.73^{A}	4.66±0.62^{A}	3.48±0.43^{A}	3.00±0.42^{A}	5.50±0.60^{ADE}
	Oral	14.60±0.86^{d}	10.18±1.04^{da}	7.48±0.22^{a}	5.52±0.29^{a}	4.32±0.17^{a}	5.14±0.40^{abef}

Results are expressed as Means ± Standard Deviation for five mice per group. Means accompanied by similar upper case letters and similar lower case letters in the same column are significantly different at $\rho \leq 0.05$ by ANOVA and post ANOVA (Bonferroni-Holm) test. Means for IP administration: $^{C}\rho < 0.05$ with respect to normal control; $^{A}\rho < 0.05$ with respect to negative control; $^{B}\rho < 0.05$ with respect to positive control; $^{D}\rho < 0.05$ with respect to 25 mg/kg body weight; $^{E}\rho < 0.05$ with respect to 48.4 mg/kg body weight; $^{F}\rho < 0.05$ with respect to 93.5 mg/kg body weight; $^{G}\rho < 0.05$ with respect to 180 mg/kg body weight; $^{H}\rho < 0.05$ with respect to 350 mg/kg body weight. Means for Oral administration: $^{d}\rho < 0.05$ with respect to normal control; $^{a}\rho < 0.05$ with respect to negative control; $^{b}\rho < 0.05$ with respect to positive control $^{e}\rho < 0.05$ with respect to 25 mg/kg body weight; $^{f}\rho < 0.05$ with respect to 48.4 mg/kg body weight; $^{g}\rho < 0.05$ with respect to 93.5 mg/kg body weight; $^{h}\rho < 0.05$ with respect to 180 mg/kg body weight; $^{i}\rho < 0.05$ with respect to 350 mg/kg body weight. Value followed by $^{*}p < 0.05$ is considered statistically significant when the mean of the oral group is compared to intraperitoneal group by T-test.

Table 4.8: Effects of administration of five therapeutic doses of aqueous plant extracts of *Vernonia lasiopus* at different times on blood glucose levels in alloxan induced diabetic mice

Treatment	Route	Levels of Glucose at Varying Times (mmol/L)					
		0 hour	2 hour	4 hour	6 hour	8 hour	24 hour
Normal control	IP	5.18±0.20	5.20±0.03	5.16±0.02	5.18±0.04	5.16±0.02	5.24±0.02
	Oral	5.18±0.02	5.20±0.03	5.16±0.02^{ghi}	5.18±0.04^{befghi}	5.16±0.02^{befghi}	5.24±0.02^{hi}
Negative control	IP	13.90±1.28^{kmnop}	15.10±1.29^{kmnop}	16.42±1.10^{kmnop}	17.84±1.16^{kmnopq}	19.06±1.13^{kmnopq}	22.62±1.06^{opq}
	Oral	13.42±1.32^{JLNQR}	15.12±1.20^{JLNQR}	17.42±1.11^{JLNQR}	18.84±1.18^{JLNQRS}	19.08±1.14^{JLNQRS}	22.80±1.12^{QRS}
Positive control (insulin)	IP	15.78±1.26	6.46±0.29^{mnopq}	5.86±0.24^{mnopq}	5.62±0.20^{mnopq}	5.20±0.14^{mnopq}	6.90±0.16^{mnopq}
Positive control (glibenclamide)	Oral	16.42±1.38	10.92±0.96^{LNS}	8.04±0.45^{LNQRS}	6.28±0.34^{NQRS}	5.20±0.11^{NQRS}	8.18±0.53^{LNQRS}
Extract dose (mg/kg body weight)							
25	IP	13.88±0.48	7.20±0.55^{mn}	5.70±0.24^{mnop}	4.48±0.13^{nop}	4.14±0.09^{nop}	9.84±0.38
	Oral	13.98±1.29	10.44±0.82^{LNS}	7.90±0.55^{LNQRS}	6.80±0.44^{NQRS}	5.98±0.48^{NQR}	9.78±0.66^{LNQS}
48.4	IP	14.24±0.84	5.60±0.48^{mn}	4.54±0.46^{nop}	3.36±0.26^{nop}	2.98±0.22^{nop}	8.02±0.12^{m}
	Oral	14.44±0.96	9.08±0.43^{LS}	6.50±0.39^{NQS}	4.70±0.21^{NQR}	4.04±0.26^{QR}	8.20±0.40^{LNS}
93.5	IP	14.10±0.23	6.22±0.28^{mnq}	5.26±0.27^{mno}	4.30±0.32^{nop}	3.72±0.18^{op}	7.30±0.29^{mq}
	Oral	19.44±0.64	9.70±0.58^{LS}	6.74±0.72^{NQS}	4.48±0.37^{NQR}	3.66±0.21^{QR}	7.90±0.36^{LNS}
180.9	IP	13.16±0.67	6.48±0.70^{mnq}	4.74±0.62^{mnopq}	3.44±0.29^{nop}	2.88±0.32^{nop}	6.94±0.20^{mnq}
	Oral	14.78±0.83	8.86±0.45^{LNS}	7.12±0.36^{LNQS}	5.72±0.42^{NQRS}	4.56±0.45^{QR}	7.16±0.22^{LNQS}
350	IP	12.66±0.71	6.64±0.73^{mnq}	4.66±0.62^{mnopq}	3.48±0.43^{nopq}	3.00±0.42^{nopq}	5.50±0.60^{mnopq}
	Oral	14.60±0.86	10.18±1.04^{LN}	7.48±0.22^{LNQS}	5.52±0.29^{NQRS}	4.32±0.17^{QRS}	5.14±0.40^{NQRS}

Results are expressed as Means ± Standard Deviation for fivemice per group. Means accompanied by similar upper case letters and similar lower case letters in the same row are not significantly different at $\rho \leq 0.05$ by ANOVA and post ANOVA (Bonferroni-Holm) test.

Means for IP administration: $^{k}\rho < 0.05$ with respect to 0 hr; $^{m}\rho < 0.05$ with respect to 2 hr; $^{n}\rho < 0.05$ with respect to 4 hr; $^{o}\rho < 0.05$ with respect to 6 hr; $^{p}\rho < 0.05$ with respect to 8 hr; $^{q}\rho < 0.05$ with respect to 24 hr. Means for Oral administration: $^{J}\rho < 0.05$ with respect to 0 hr; $^{L}\rho < 0.05$ with respect to 2 hr; $^{N}\rho < 0.05$ with respect to 4 hr; $^{Q}\rho < 0.05$ with respect to 6 hr; $^{R}\rho < 0.05$ with respect to 8 hr; $^{S}\rho < 0.05$ with respect to 24 hr.

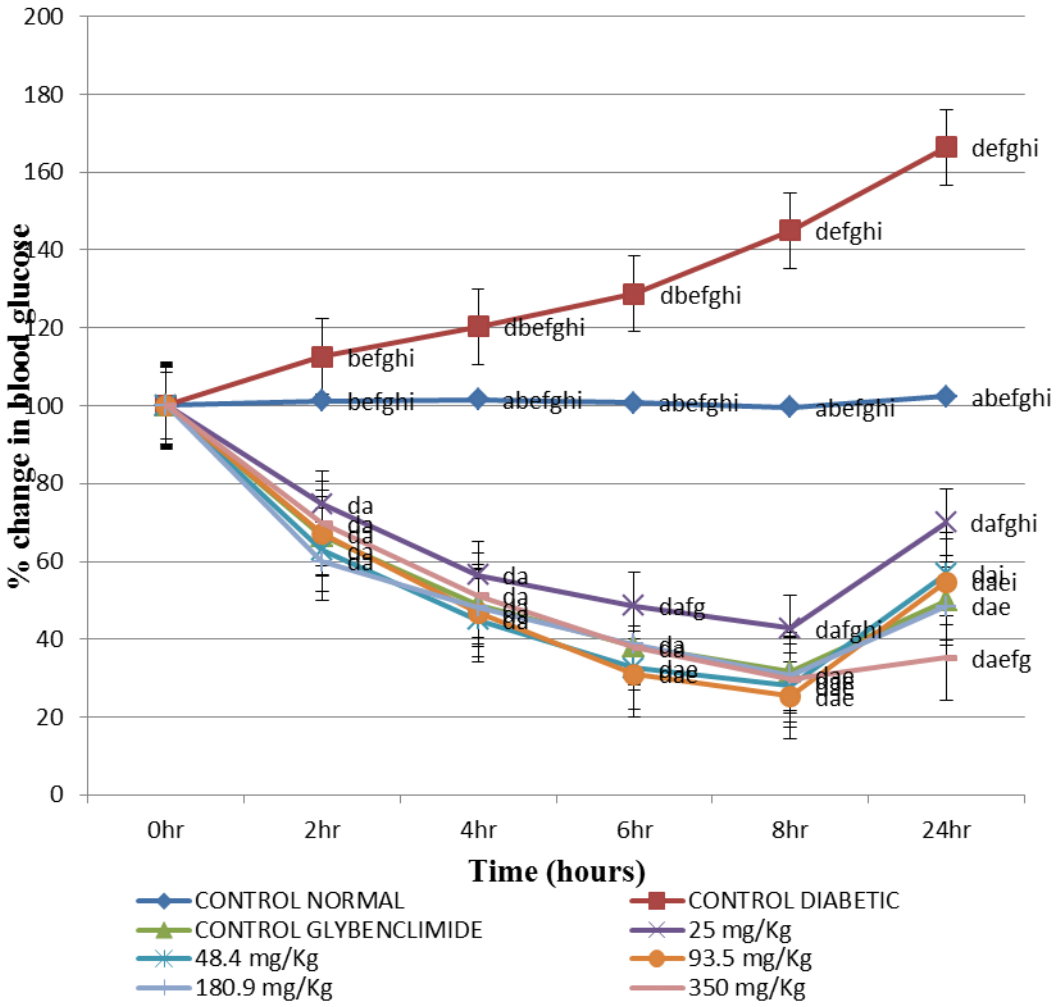

Figure 4.5: Percentage reduction in blood glucose levels at varying times after oral administration of the aqueous extracts of *Vernonia lasiopus* in alloxan induced diabetic male mice.

Values are expressed as % means ± SEM for five animals for each time point. $^{d}\rho < 0.05$ when compared to normal control; $^{a}\rho < 0.05$ when compared to negative control; $^{b}\rho < 0.05$ when compared to positive control; $^{e}\rho < 0.05$ when compared to 25 mg/kg body weight; $^{f}\rho < 0.05$ when compared to 48.4 mg/kg body weight; $^{g}\rho < 0.05$ when compared to 93.5 mg/kg body weight; $^{h}\rho < 0.05$ when compared to 180 mg/kg body weight; $^{i}\rho < 0.05$ when compared to 350 mg/kg body weight.

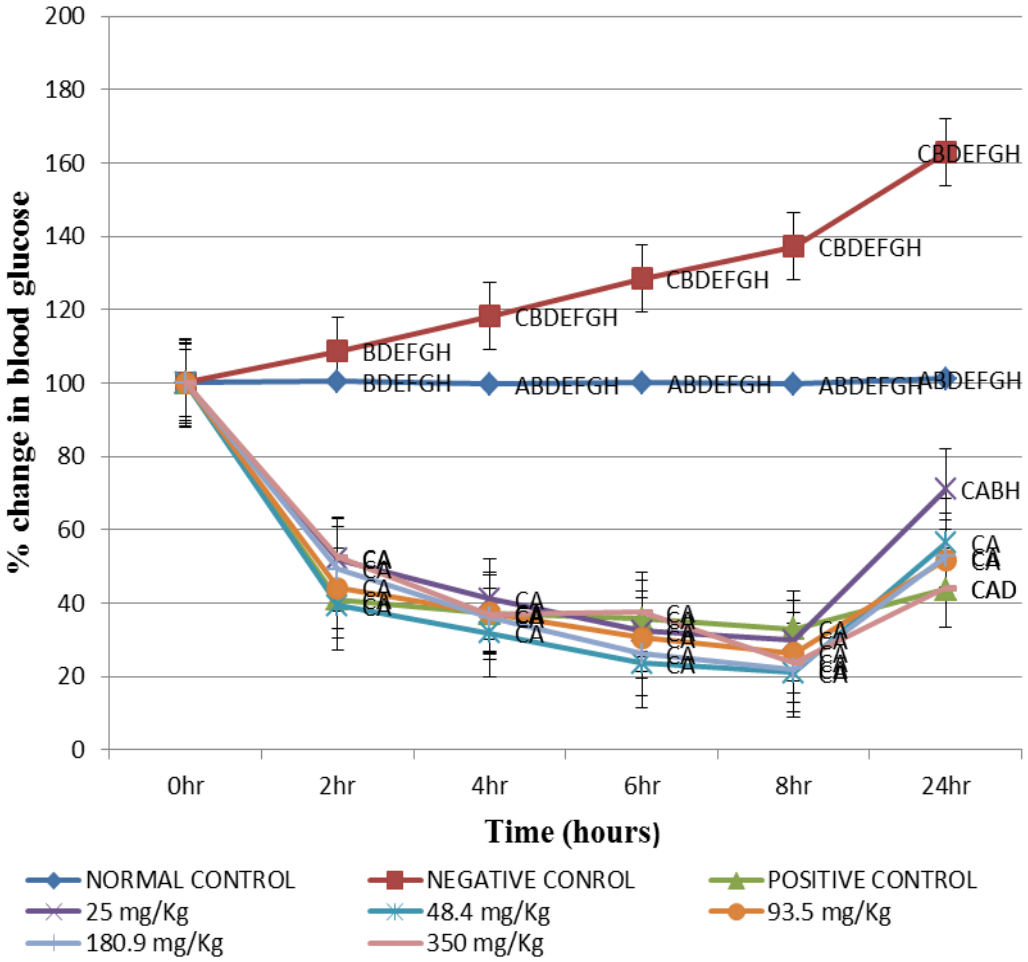

Figure 4.6: Percentage reduction in blood glucose levels at varying times after intraperitoneal administration of the aqueous extracts of *Vernonia lasiopus* in alloxan induced diabetic male mice.
Values are expressed as % means ± SEM for five animals for each time point. $^{C}\rho < 0.05$ when compared to normal control; $^{A}\rho < 0.05$ when compared to negative control; $^{B}\rho < 0.05$ when compared to positive control; $^{D}\rho < 0.05$ when compared to 25 mg/kg body weight; $^{E}\rho < 0.05$ when compared to 48.4 mg/kg body weight; $^{F}\rho < 0.05$ when compared to 93.5 mg/kg body weight; $^{G}\rho < 0.05$ when compared to 180 mg/kg body weight; $^{H}\rho < 0.05$ when compared to 350 mg/kg body weight.

Table 4.9 shows the pharmacokinetics of the hypoglycemic activity for the first four hours of the aqueous leaf extracts of *V. lasiopus*. Results indicate that the pseudo-first order rate constants for the orally administered doses of the aqueous extracts of *V. lasiopus* at 25, 48.4, 93.5, 180.9 and 350 mg/kg were 0.2854, 0.3991, 0.381, 0.3652 and 0.3344, respectively and their accompanying half-lifes were 2.43, 1.74, 1.82, 1.90, and 2.07, respectively. The half-life of the 25mg/kg dose is

higher while those of the other doses were lower than that of glibenclamide. Pseudo-first order rate constants for the intraperitoneally administered doses of aqueous extracts of *V. lasiopus* at 25, 48.4, 93.5, 180.9 and 350 mg/kg were 0.445, 0.4152, 0.493, 0.5106 and 0.5998, respectively and their accompanying half-lifes were 1.56, 1.67, 1.41, 1.36 and 1.16, respectively. The half-lifes of 25 mg/kg, and 48.4 mg/kg doses were higher than that of insulin while those of the other doses were lower. The rate constants for the orally administered aqueous extracts for the five doses ranged from 0.2854 to 0.3991 per hour and the half-lifes ranged from 2.43 to 1.74 hours, respectively. Rate constants for the intraperitoneally administered aqueous extracts for the five doses ranged from 0.4152 to 0.5998 per hour and the half-lifes ranged from 1.67 to 1.16 hours, respectively. The rate constant for insulin was 0.4953 per hour and that of glibenclamide was 0.3069 per hour while their corresponding half-lifes were 1.40 and 2.26 hours, respectively.

Table 4.9: Pharmacokinetics of the hypoglycemic activity for the first four hours of the five doses of the aqueous extracts of *V. lasiopus*

Drug (dose)	Route	Rate constant ($hour^{-1}$)	Half-life (hours)
Insulin	IP	0.4953	1.40
Glibenclamide	Oral	0.3069	2.26
Extract (mg/kg bw)			
25	IP	0.4450	1.56
	Oral	0.2854	2.43
48.4	IP	0.4152	1.67
	Oral	0.3991	1.74
93.4	IP	0.4930	1.41
	Oral	0.3810	1.82
180.9	IP	0.5106	1.36
	Oral	0.3652	1.90
350	IP	0.5998	1.16
	Oral	0.3344	2.07

Results are expressed as Means of five mice for each time point; bw represents body weight

4.1.4 Effects of administration of *Galinsoga paviflora* extracts on blood glucose levels in male diabetic mice

Aqueous extract of plant extract of *Galinsoga parviflora* yielded a black lyophilate whose concentration was 95 mg/g dry weight. Oral administration of aqueous extracts of *Galinsoga paviflora* at 25, 48.4, 93.5, 180.9, and 350 mg/kg body weight to mice decreased the blood sugar levels significantly as from the 2nd hour independent of the dose. During the 2nd hour, the percentage reductions of blood glucose levels in diabetic mice by the aqueous extract of *Galinsoga paviflora* at 25, 48.4, 93.5, 180.9, and 350 mg/kg body weight was 76.1, 64.5, 68.9, 61.3 and 76.2%, respectively, compared to glibenclamide treated diabetic mice whose blood sugar levels was lowered to 70.7% (Figure 4.7). At this hour, the five aqueous extracts lowered the blood glucose levels in diabetic mice but not to normal levels ($^{\mathbf{d}}\rho < 0.05$) (Table 4.10); however, the five aqueous extracts doses of *Galinsoga paviflora* significantly lowered blood glucose levels in diabetic mice relative to the diabetic control mice ($^{\mathbf{a}}\rho < 0.05$) (Table 4.10).

In the 4th hour, the glucose lowering effect by the five extracts doses of *Galinsoga paviflora* in diabetic mice was also observed; the percentage reduction of blood glucose was 55.9, 44.6, 51.0, 43.6 and 58.8%, respectively, compared to glibenclamide treated diabetic mice whose blood sugar levels was lowered to 54.1%. By the 6th hour, the five extracts doses of *Galinsoga paviflora* lowered blood sugar levels in diabetic mice to 46.6, 32.7, 33.8, 31.8 and 40.0%, respectively, compared to glibenclamide-treated diabetic mice whose blood sugar levels was lowered to 41.6%. The five extracts doses of *Galinsoga paviflora* lowered blood glucose in diabetic mice to normal ($^{\mathbf{d}}\rho < 0.05$). At this hour, the five extract doses of *Galinsoga paviflora* lowered blood glucose levels in diabetic mice

as effectively as glibenclamide ($^{b}\rho < 0.05$). During the 8th hour, the same trend in blood glucose lowering in diabetic mice treated with the five aqueous extracts doses of *Galinsoga paviflora* was lower than that of glibenclamide (34.3%). The blood glucose lowering in diabetic mice treated with the five aqueous extracts doses were 40.4, 27.1, 25.5, 25.0 and 31.9%, respectively (Figure 4.7). In the 24th hour, the blood glucose level in diabetic mice treated with aqueous extracts of *Galinsoga parviflora* at 25, 48.4 and 93.5 mg/kg body weight had returned to the diabetic state but those treated with 180.9 and 350 mg/kg body weight dose had not returned to the diabetic state (Table 10).

Intraperitoneal administration of aqueous extracts of *Galinsoga parviflora* at 25, 48.4, 93.5, 180.9, and 350 mg/kg body weight to mice decreased the blood sugar levels significantly as from the 2nd hour independent of the dose. During the 2nd hour, the percentage reduction in blood glucose levels in diabetic mice by the aqueous extract of *Galinsoga parviflora* at 25, 48.4, 93.5, 180.9, and 350 mg/kg body weight was 54.9, 43.7, 47.5, 52.0 and 47.1%, respectively, compared to insulin-treated diabetic mice whose blood sugar levels was lowered to 43.2% (Figure 4.8). At this hour, the five aqueous extracts doses lowered the blood glucose levels in diabetic mice but not to normal levels ($^{C}\rho < 0.05$) (Table 4.10). The five extract doses, however, significantly lowered blood glucose levels in diabetic mice relative to the diabetic control mice ($^{A}\rho < 0.05$) (Table 4.10).

In the 4th hour, the glucose lowering effect by the five dose levels in diabetic mice was also observed; the percentage reduction of blood glucose was 31.8, 38.8, 36.3, 42.0 and 35.1%, respectively, compared to insulin-treated diabetic mice whose

blood sugar levels was lowered to 38.9%. The five extract doses lowered blood glucose in diabetic mice to normal levels ($^{C}\rho > 0.05$). By the 6th hour, the five extract doses lowered blood sugar levels in diabetic mice to 28.9, 27.4, 32.5, 35.4 and 29.1%, respectively, compared to insulin-treated diabetic mice whose blood sugar levels was lowered to 34.3%. At this hour, the five extract doses lowered blood glucose levels in diabetic mice as effectively as insulin ($^{B}\rho < 0.05$) especially the 93.5 mg/kg body weight dose. During the 8th hour, the same trend in blood glucose lowering by the five extracts doses of *Galinsoga parviflora* in diabetic mice was 25.8, 24.7, 27.5, 31.0 and 23.5%, respectively, which was lower than that of insulin (31.9%) (Figure 4.8). At the 24th hour, the blood glucose levels in diabetic mice administered with aqueous extracts of *Galinsoga parviflora* at 93.5, 180.9 and 350 mg/kg body weight doses had not returned to the diabetic state but those treated with aqueous extracts of *Galinsoga parviflora* at 25 and 48.4 mg/kg body weight doses were still diabetic (Table 10). The oral and intraperitoneal administration of aqueous extracts of *Galinsoga parviflora* at all the tested doses lowered blood glucose in diabetic mice to similar levels (Table 4.10).

Table 4.10: Effects of administration of five therapeutic doses of aqueous extracts of *Galinsoga paviflora* at different times on blood glucose levels in alloxan induced diabetic mice

Treatment	Route	Levels of Glucose at Varying Times (mmol/L)					
		0 hour	2 hour	4 hour	6 hour	8 hour	24 hour
Normal control	IP	5.36±0.04^{ABEFGH}	5.40±0.05^{A}	5.38±0.06^{A}	5.34±0.05^{A}	5.28±0.04^{AFH}	5.32±0.06^{ABE}
	Oral	5.24±0.02abefghi	5.24±0.05^{abefgi}	5.22±0.04^{ai}	5.18±0.04^{a}	5.26±0.04^{agh}	5.22±0.04^{abefg}
Negative control	IP	14.44±0.82^{C}	15.72±0.92^{CBDEFG}	16.60±0.86^{CBDEFG}	17.88±0.91^{CBDEFG}	19.32±0.81^{CBDEFG}	22.26±1.11^{CBDEFG}
	Oral	17.76±0.74^{d}	19.44±0.79^{dbefgh}	21.30±1.11^{dbefgh}	23.38±0.84^{dbefgh}	25.54±0.64^{dbefgh}	26.96±0.63^{dbefgh}
Positive control (insulin)	IP	15.00±0.78^{C}	6.48±0.25^{A}	5.84±0.24^{A}	5.14±0.15^{A}	4.78±0.16^{A}	7.38±0.23^{A}
Positive control (glibenclamide)	Oral	15.52±0.98^{d}	10.26±1.23^{da}	7.86±0.60^{a}	6.04±0.37^{a}	4.98±0.14^{ag}	7.78±0.04^{di}
Extract dose (mg/kg body weight)							
25	IP	13.78±0.40^{C}	7.56±0.94^{A}	4.38±0.21^{A}	3.98±0.20^{A}	3.56±0.17^{A}	8.46±0.36^{CAFH}
	Oral	13.66±1.05^{d}	10.40±1.20^{da}	7.64±0.37^{a}	6.36±0.41^{a}	5.52±0.27^{afghi}	9.56±0.26^{dghi}
48.4	IP	13.22±0.94^{C}	5.78±0.63^{A}	4.14±0.26^{A}	3.62±0.22^{A}	3.26±0.21^{CA}	7.94±0.55^{CAH}
	Oral	14.48±0.70^{d}	9.48±0.47^{da}	6.46±0.40^{ia}	4.74±0.20^{a}	3.92±0.32^{ae}	8.42±0.46^{dai}
93.5	IP	12.88±0.65^{C}	6.12±0.77^{A}	4.68±0.65^{A}	4.18±0.45^{A}	3.54±0.31^{CA}	7.32±0.30^{A}
	Oral	13.44±0.65^{da}	9.26±0.34^{da}	6.86±0.58^{a}	4.54±0.18^{a}	3.42±0.24^{dabe}	7.52±0.38^{daefi}
180.9	IP	13.34±0.43^{C}	6.94±0.75^{A}	5.60±0.36^{A}	4.72±0.36^{A}	4.14±0.40^{A}	6.76±0.48^{A}
	Oral	14.26±1.27^{d}	8.74±0.56^{a}	6.22±0.66^{a}	4.54±0.46^{a}	3.56±0.26^{dae}	6.96±0.49^{aei}
350	IP	13.26±0.50^{C}	6.24±0.72^{A}	4.66±0.34^{A}	3.86±0.29^{A}	3.12±0.14^{CA}	5.30±0.31^{CADE}
	Oral	13.92±0.84^{d}	10.10±0.57^{da}	8.18±0.42^{da}	5.58±0.40^{a}	4.44±0.29^{a}	4.58±0.35^{abefgh}

Results are expressed as Means ± Standard Deviation for five mice per group. Means accompanied by similar upper case letters and similar lower case letters in the same column are significantly different at $\rho \leq 0.05$ by ANOVA and post ANOVA (Bonferroni-Holm) test.

Means for IP administration: $^{C}\rho < 0.05$ with respect to normal control; $^{A}\rho < 0.05$ with respect to negative control; $^{B}\rho < 0.05$ with respect to positive control; $^{D}\rho < 0.05$ with respect to 25 mg/kg body weight; $^{E}\rho < 0.05$ with respect to 48.4 mg/kg body weight; $^{F}\rho < 0.05$ with respect to 93.5 mg/kg body weight; $^{G}\rho < 0.05$ with respect to 180 mg/kg body weight; $^{H}\rho < 0.05$ with respect to 350 mg/kg body weight. Means for Oral administration: $^{d}\rho < 0.05$ with respect to normal control; $^{a}\rho < 0.05$ with respect to negative control; $^{b}\rho < 0.05$ with respect to positive control $^{e}\rho < 0.05$ with respect to 25 mg/kg body weight; $^{f}\rho < 0.05$ with respect to 48.4 mg/kg body weight; $^{g}\rho < 0.05$ with respect to 93.5 mg/kg body weight; $^{h}\rho < 0.05$ with respect to 180 mg/kg body weight; $^{i}\rho < 0.05$ with respect to 350 mg/kg body weight. Value followed by $*\rho < 0.05$ is considered statistically significant when the mean of the oral group is compared to intraperitoneal group by T-test.

Table 4.11: Effects of administration of five therapeutic doses of aqueous plant extracts of *Galinsoga paviflora* at different times on blood glucose levels in alloxan induced diabetic mice

Treatment	Route	Levels of Glucose at Varying Times (mmol/L)					
		0 hour	2 hour	4 hour	6 hour	8 hour	24 hour
Normal control	IP	5.36±0.04	5.40±0.05	5.38±0.06	5.34±0.05	5.28±0.04	5.32±0.06
	Oral	5.24±0.02	5.24±0.05^{h}	5.22±0.04^{befgh}	5.18±0.04^{befghi}	5.26±0.04^{befi}	5.22±0.04^{hi}
Negative control	IP	14.44±0.82^{kmno}	15.72±0.92^{kmnop}	16.60±0.86^{kmnop}	17.88±0.91^{kmnop}	19.32±0.81^{mnopq}	22.26±1.11^{pq}
	Oral	17.76±0.74^{JLN}	19.44±0.79^{JLN}	21.30±1.11^{JLNQ}	23.38±0.84^{NQRS}	25.54±0.64^{QRS}	26.96±0.63^{QRS}
Positive control (insulin)	IP	15.00±0.78	6.48±0.25^{mnopq}	5.84±0.24^{mnopq}	5.14±0.15^{mnop}	4.78±0.16^{mnop}	7.38±0.23^{mnq}
Positive control (glibenclamide)	Oral	15.52±0.98	10.26±1.23^{LNS}	7.86±0.60^{LNQRS}	6.04±0.37^{NQRS}	4.98±0.14^{NQRS}	7.78±0.04^{LNQRS}
Extract dose (mg/kg body weight)							
25	IP	13.78±0.40	7.56±0.94^{mq}	4.38±0.21^{nop}	3.98±0.20^{nop}	3.56±0.17^{nop}	8.46±0.36^{mq}
	Oral	13.66±1.05	10.40±1.20^{LNS}	7.64±0.37^{LNQRS}	6.36±0.41^{NQRS}	5.52±0.27^{NQR}	9.56±0.26^{LNQS}
48.4	IP	13.22±0.94	5.78±0.63^{mnoq}	4.14±0.26^{mnop}	3.62±0.22^{mnop}	3.26±0.21^{nop}	7.94±0.55^{mq}
	Oral	14.48±0.70	9.48±0.47^{LS}	6.46±0.40^{NQS}	4.74±0.20^{NQR}	3.92±0.32^{QR}	8.42±0.46^{LNS}
93.5	IP	12.88±0.65	6.12±0.77^{mnoq}	4.68±0.65^{mnop}	4.18±0.45^{mnop}	3.54±0.31^{nop}	7.32±0.30^{mq}
	Oral	13.44±0.65	9.26±0.34^{LS}	6.86±0.58^{NS}	4.54±0.18^{QR}	3.42±0.24^{QR}	7.52±0.38^{LNS}
180.9	IP	13.34±0.43	6.94±0.75^{mnoq}	5.60±0.36^{mnopq}	4.72±0.36^{mnopq}	4.14±0.40^{nop}	6.76±0.48^{mnoq}
	Oral	14.26±1.27	8.74±0.56^{LNS}	6.22±0.66^{LNQRS}	4.54±0.46^{NQRS}	3.56±0.26^{NQR}	6.96±0.49^{LNQS}
350	IP	13.26±0.50	6.24±0.72^{mnq}	4.66±0.34^{mnopq}	3.86±0.29^{nopq}	3.12±0.14^{nop}	5.30±0.31^{mnoq}
	Oral	13.92±0.84	10.10±0.57^{LN}	8.18±0.42^{LN}	5.58±0.40^{QRS}	4.44±0.29^{QRS}	4.58±0.35^{QRS}

Results are expressed as Means ± Standard Deviation for five mice per group. Means accompanied by similar upper case letters and similar lower case letters in the same row are not significantly different at $\rho \leq 0.05$ by ANOVA and post ANOVA (Bonferroni-Holm) test.

Means for IP administration: $^{k}\rho < 0.05$ with respect to 0 hr; $^{m}\rho < 0.05$ with respect to 2 hr; $^{n}\rho < 0.05$ with respect to 4 hr; $^{o}\rho < 0.05$ with respect to 6 hr; $^{p}\rho < 0.05$ with respect to 8 hr; $^{q}\rho < 0.05$ with respect to 24 hr. Means for Oral administration: $^{J}\rho < 0.05$ with respect to 0 hr; $^{L}\rho < 0.05$ with respect to 2 hr; $^{N}\rho < 0.05$ with respect to 4 hr; $^{Q}\rho < 0.05$ with respect to 6 hr; $^{R}\rho < 0.05$ with respect to 8 hr; $^{S}\rho < 0.05$ with respect to 24 hr.

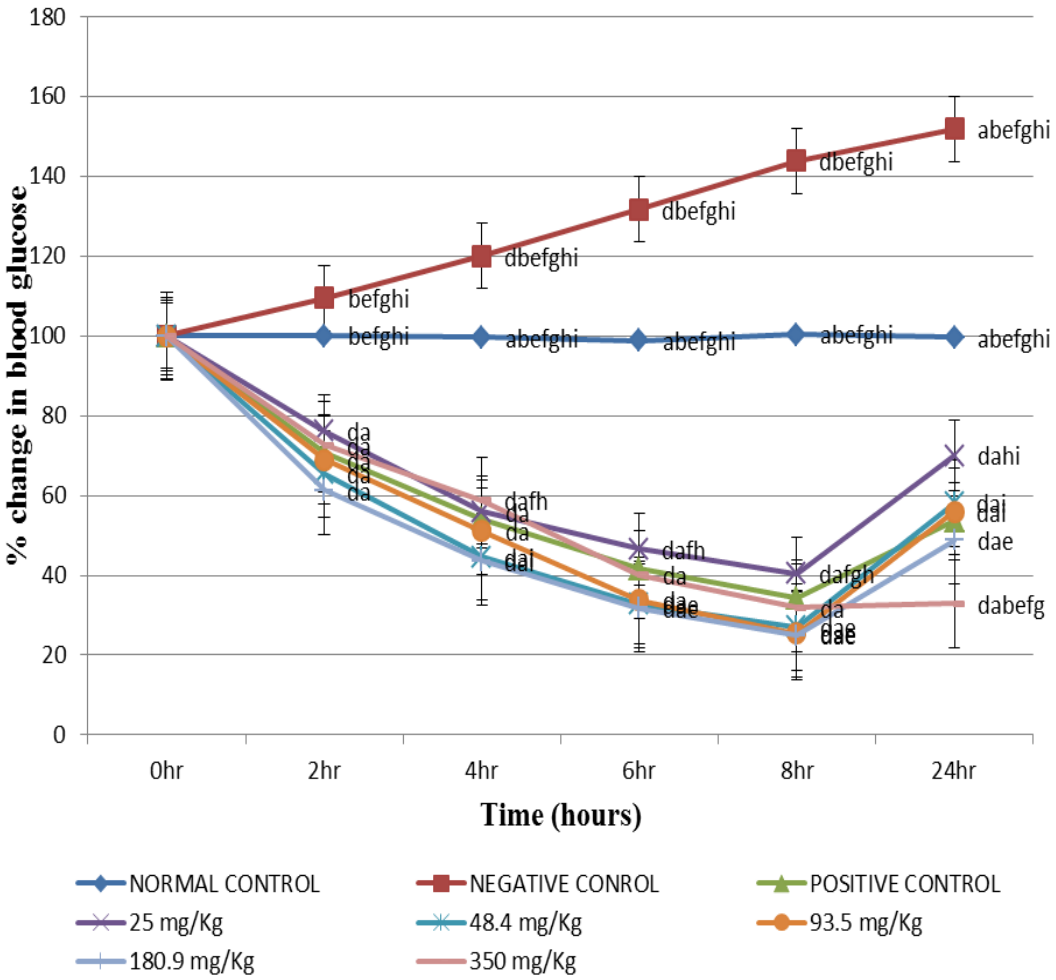

Figure 4.7: Percentage reduction in blood glucose levels at varying times after oral administration of the aqueous extracts of *Galinsoga paviflora* in alloxan induced diabetic male mice.

Values are expressed as % means ± SEM for five animals for each time point. $^{d}\rho < 0.05$ when compared to normal control; $^{a}\rho < 0.05$ when compared to negative control; $^{b}\rho < 0.05$ when compared to positive control; $^{e}\rho < 0.05$ when compared to 25 mg/kg body weight; $^{f}\rho < 0.05$ when compared to 48.4 mg/kg body weight; $^{g}\rho < 0.05$ when compared to 93.5 mg/kg body weight; $^{h}\rho < 0.05$ when compared to 180 mg/kg body weight; $^{i}\rho < 0.05$ when compared to 350 mg/kg body weight.

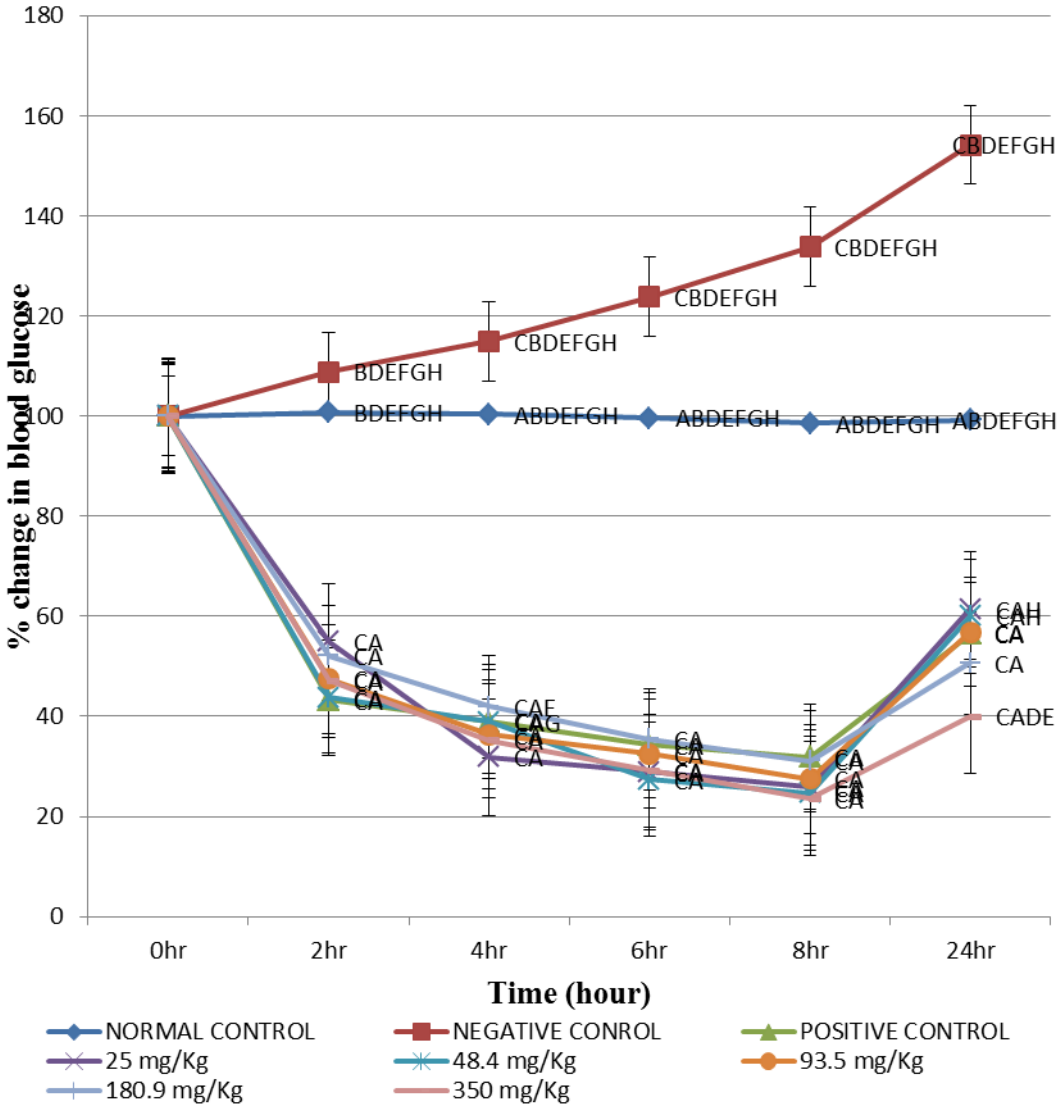

Figure 4.8: Percentage reduction in blood glucose levels at varying times after intraperitoneal administration of the aqueous extracts of *Galinsoga parviflora* in alloxan induced diabetic male mice.
Values are expressed as % means ± SEM for five animals for each time point. $^{C}\rho < 0.05$ when compared to normal control; $^{A}\rho < 0.05$ when compared to negative control; $^{B}\rho < 0.05$ when compared to positive control; $^{D}\rho < 0.05$ when compared to 25 mg/kg body weight; $^{E}\rho < 0.05$ when compared to 48.4 mg/kg body weight; $^{F}\rho < 0.05$ when compared to 93.5 mg/kg body weight; $^{G}\rho < 0.05$ when compared to 180 mg/kg body weight; $^{H}\rho < 0.05$ when compared to 350 mg/kg body weight.

Table 4.12 shows the pharmacokinetics of the hypoglycemic activity for the first four hours of the aqueous leaf extracts of *G. parviflora.* Results indicate that the pseudo-first order rate constants for the orally administered doses of the aqueous extracts of *G. parviflora* at 25, 48.4, 93.5, 180.9 and 350 mg/kg body weight doses

were 0.2905, 0.4036, 0.3363, 0.4148 and 0.2658, respectively, and their accompanying half-lifes were 2.39, 1.72, 2.06, 1.67, and 2.61 hours, respectively. The half-life of 25 and 350 mg/kg body weight doses were higher while those of the other doses were lower than that of glibenclamide. Pseudo-first order rate constants for the intraperitoneally administered doses of aqueous extracts of *G. parviflora* at 25, 48.4, 93.5, 180.9 and 350 mg/kg body weight were 0.5731, 0.5805, 0.5062, 0.434 and 0.5229, respectively, and their accompanying half-lifes were 1.21, 1.19, 1.37, 1.60 and 1.33 hours, respectively. The half-livee of 180.9 mg/kg body weight dose was higher than that of insulin while those of the other doses were lower. The rate constants for the orally administered aqueous extracts of the five doses ranged from 0.2658 to 0.4148 per hour and the half-lives ranged from 2.61 to 1.67 hours, respectively.

Rate constants for the intraperitoneally administered aqueous extracts for the five doses ranged from 0.434 to 0.5805 per hour and the half-lives ranged from 1.60 to 1.19 hours, respectively. The rate constant for insulin was 0.4717 per hour and that of glibenclamide was 0.3069 per hour while their corresponding half-lives were 1.47 and 2.26 hours, respectively.

Table 4.12: Pharmacokinetics of the hypoglycemic activity for the first four hours of the five doses of the aqueous extracts of *G. parviflora*

Drug (dose)	Route	Rate constant ($hour^{-1}$)	Half-life (hours)
Insulin	IP	0.4712	1.47
Glibenclamide	Oral	0.3069	2.26
Extract (mg/kg bw)			
25	IP	0.5731	1.21
	Oral	0.2905	2.39
48.4	IP	0.5805	1.19
	Oral	0.4036	1.72
93.4	IP	0.5062	1.37
	Oral	0.3363	2.06
180.9	IP	0.4340	1.60
	Oral	0.4148	1.67
350	IP	0.5229	1.33
	Oral	0.2658	2.61

Results are expressed as Means of five mice for each time point; bw represents body weight

4.1.5 Effects of administration of *Sonchus asper* extracts on blood glucose levels in male diabetic mice

Sonchus asper aqueous extracts yielded a dark green paste of concentration 67.5 mg/g dry weight. Oral administration of aqueous extracts of *Sonchus asper* at 25, 48.4, 93.5, 180.9, and 350 mg/kg body weight to mice decreased the blood sugar levels significantly as from the 2nd hour independent of the dose. During the 2nd hour, the percentage reduction of blood glucose levels in diabetic mice by the aqueous extract of *Sonchus asper* at 25, 48.4, 93.5, 180.9, and 350 mg/kg body weight was 74.9, 67.2, 78.2, 75.8 and 78.4%, respectively, compared to glibenclamide-treated diabetic mice whose blood sugar levels was lowered to 78.4% (Figure 4.9). At this hour, the five aqueous extracts of *Sonchus asper* administered to diabetic mice lowered the blood glucose levels but not to normal levels ($^{\mathbf{d}}\rho < 0.05$) (Table 4.13). The five aqueous doses, however, significantly lowered blood glucose levels in diabetic mice relative to the diabetic control mice ($^{\mathbf{a}}\rho < 0.05$) (Table 4.13).

In the 4th hour, the glucose lowering effect by the five aqueous extracts doses of *Sonchus asper* in diabetic mice was also observed; the percentage reduction of blood glucose in diabetic mice was 57.1, 60.9, 61.1, 58.4 and 57.8%, respectively, compared to glibenclamide-treated diabetic mice whose blood sugar levels was lowered to 59.4%. By the 6th hour, the five aqueous extracts doses of *Sonchus asper* lowered blood sugar levels in diabetic mice to 45.2, 48.4, 45.8, 44.0 and 45.7%, respectively, compared to glibenclamide-treated diabetic mice whose blood sugar levels was lowered to 43.7%. The five aqueous extracts of *Sonchus asper* lowered blood glucose levels to normal at this hour ($^{d}\rho > 0.05$). At this hour, the five aqueous extracts doses of *Sonchus asper* lowered blood glucose levels as effectively as glibenclamide ($^{b}\rho < 0.05$) especially by the 180.9 mg/kg body weight dose. The same trend was observed during the 8th hour where the five dose levels of *Sonchus asper* lowered blood glucose levels to 39.4, 38.2, 37.4, 37.3 and 37.8%, respectively, compared to glibenclamide-treated diabetic mice whose blood sugar levels was lowered to 35.4% (Figure 4.9). The mice treated with with orally administered extracts of *Sonchus asper* at 25, 48.4, 93.5 and 180.9 mg/kg body weight doses returned to the diabetic state at the 24th hour but those administered with 350mg/kg had not (Table 4.13).

Intraperitoneal administration of aqueous extracts of *Sonchus asper* at 25, 48.4, 93.5, 180.9, and 350 mg/kg body weight to mice decreased the blood sugar levels significantly as from the 2nd hour independent of the dose. During the 2nd hour, the percentage reduction of blood glucose levels in diabetic mice by the five aqueous extracts of *Sonchus asper* at 25, 48.4, 93.5, 180.9, and 350 mg/kg body weight was 72.5, 64.0, 69.3, 72.3 and 70.3%, respectively, compared to insulin-treated diabetic

mice whose blood sugar levels was lowered to 45.4%. At this hour, the aqueous extracts of *Sonchus asper* lowered the blood glucose levels but not to normal levels ($^{C}\rho < 0.05$) (Table 4.13). The five aqueous extracts doses of *Sonchus asper*, however, significantly lowered blood glucose levels relative to the diabetic control mice ($^{A}\rho < 0.05$) (Table 4.13).

In the 4th hour, the glucose lowering effect by the five dose levels of *Sonchus asper* was also observed; the percentage reduction of blood glucose was 52.2, 52.9, 54.2, 53.3 and 53.2%, respectively, compared to insulin-treated diabetic mice whose blood sugar levels was lowered to 42.0%. By the 6th hour, the five aqueous extracts doses of *Sonchus asper* lowered blood sugar levels to 44.8, 44.2, 44.5, 42.8 and 42.6%, respectively, compared to insulin-treated diabetic mice whose blood sugar levels was lowered to 37.2%. The five aqueous extracts doses of *Sonchus asper* lowered blood glucose levels in diabetic mice to normal at this hour ($^{C}\rho > 0.05$). At this hour, the five aqueous extracts doses of *Sonchus asper* lowered blood glucose levels in diabetic mice as effectively as insulin ($^{B}\rho < 0.05$). During the 8th hour, the same trend by five aqueous extracts doses of *Sonchus asper* was observed where blood glucose levels was lowered to 38.7, 39.8, 38.9, 36.4 and 36.3%, respectively, compared to insulin-treated diabetic mice whose blood sugar level was lowered to 34.7% (Figure 4.10).

In the 24th hour, the blood glucose levels in diabetic mice administered with aqueous extracts of *Sonchus asper* at 25, 48.4, 93.5 and 180.9 mg/kg body weight had returned to the diabetic state but those administered with aqueous extracts of *Sonchus asper* at 350 mg/kg body weight had not (Table 4.13). Oral and

intraperitoneal administration of the aqueous *Sonchus asper* at all the five aqueous extracts doses lowered blood glucose in diabetic mice to similar levels (Table 4.13).

Table 4.13: Effects of administration of five therapeutic doses of aqueous plant extracts of *Sonchus asper* at different times on blood glucose levels in alloxan induced diabetic mice

Treatment	Route	Levels of Glucose at Varying Times (mmol/L)					
		0 hour	2 hour	4 hour	6 hour	8 hour	24 hour
Normal control	IP	5.24±0.05^{ABDEFGH}	5.26±0.06^{ADEFGH}	5.24±0.04^{ADE}	5.24±0.06^{A}	5.28±0.04^{A}	5.30±0.03^{ADEFG}
	Oral	5.16±0.04^{abefgi}	5.20±0.04^{fabeghi}	5.18±0.02^{a}	5.22±0.04^{a}	5.20±0.03^{a}	5.22±0.04^{aefgh}
Negative control	IP	15.24±0.78^{C}	16.54±0.84^{CBDEFGH}	17.36±0.78^{CBDEFGH}	18.36±0.90^{CBDEFGH}	19.50±0.82^{CBDEFGH}	21.86±0.75^{CBDEFGH}
	Oral	14.94±1.17^{befghid}	16.48±1.45^{bdefghi}	17.58±1.54^{dbefghi}	19.02±1.69^{dbefghi}	20.82±1.81^{dbefghi}	22.86±1.65^{dbefghi}
Positive control (insulin)	IP	14.46±0.62^{C}	6.56±0.21^{ADG}	6.06±0.19^{A}	5.38±0.07^{A}	5.02±0.07^{A}	7.06±0.09^{ADE}
Positive control (glibenclamide)	Oral	15.18±1.59^{d}	11.90±1.80^{d}	9.02±1.21^{a}	6.64±0.65^{a}	5.38±0.22^{a}	8.60±0.70^{de}
Extract dose (mg/kg body weight)							
25	IP	15.14±0.77^{C}	10.98±0.74^{CAB}	7.90±0.43^{CA}	6.78±0.36^{A}	5.86±0.33^{A}	9.20±0.45^{CABH}
	Oral	15.06±1.06^{d}	11.28±0.55^{da}	8.60±0.49^{a}	6.80±0.18^{a}	5.94±0.29^{a}	9.18±0.61^{da}
48.4	IP	14.74±0.62^{C}	9.44±0.33^{CA}	7.80±0.25^{CA}	6.52±0.21^{A}	5.86±0.14^{A}	9.60±0.49^{CABH}
	Oral	14.62±1.08^{d}	9.82±0.94^{a}	8.90±0.67^{a}	7.08±0.38^{a}	5.58±.024^{a}	9.88±0.46^{da}
93.5	IP	13.74±059^{C}	9.52±0.44^{CA}	7.44±0.30^{A}	6.12±0.26^{A}	5.34±0.13^{A}	9.00±0.49^{CAH}
	Oral	13.42±0.86^{d}	10.50±0.67^{da}	8.20±0.53^{a}	6.14±0.47^{a}	5.02±1.59^{a}	9.30±0.48^{da}
180.9	IP	13.84±0.60^{C}	10.00±0.72^{CAB}	7.38±0.34^{A}	5.92±0.25^{A}	5.04±0.10^{A}	8.92±0.42^{CAH}
	Oral	13.56±1.11^{d}	10.28±0.62^{da}	7.92±0.40^{a}	5.96±0.27^{a}	5.06±0.06^{a}	9.04±0.67^{da}
350	IP	13.38±0.60^{C}	9.40±1.05^{CA}	7.12±0.85^{A}	5.70±0.37^{A}	4.86±0.20^{A}	6.54±0.34^{ADEFG}
	Oral	13.16±1.14^{d}	10.32±1.11^{da}	7.60±1.03^{a}	6.02±0.52^{ia}	4.96±0.14^{a}	6.64±0.32^{a}

Results are expressed as Means ± Standard Deviation for five mice per group. Means accompanied by similar upper case letters and similar lower case letters in the same column are significantly different at $\rho \leq 0.05$ by ANOVA and post ANOVA (Bonferroni-Holm) test.

Means for IP administration: $^{C}\rho < 0.05$ with respect to normal control; $^{A}\rho < 0.05$ with respect to negative control; $^{B}\rho < 0.05$ with respect to positive control; $^{D}\rho < 0.05$ with respect to 25 mg/kg body weight; $^{E}\rho < 0.05$ with respect to 48.4 mg/kg body weight; $^{F}\rho < 0.05$ with respect to 93.5 mg/kg body weight; $^{G}\rho < 0.05$ with respect to 180 mg/kg body weight; $^{H}\rho < 0.05$ with respect to 350 mg/kg body weight. Means for Oral administration: $^{d}\rho < 0.05$ with respect to normal control; $^{a}\rho < 0.05$ with respect to negative control; $^{b}\rho < 0.05$ with respect to positive control; $^{e}\rho < 0.05$ with respect to 25 mg/kg body weight; $^{f}\rho < 0.05$ with respect to 48.4 mg/kg body weight; $^{g}\rho < 0.05$ with respect to 93.5 mg/kg body weight; $^{h}\rho < 0.05$ with respect to 180 mg/kg body weight; $^{i}\rho < 0.05$ with respect to 350 mg/kg body weight. Value followed by $^{*}p < 0.05$ is considered statistically significant when the mean of the oral group is compared to intraperitoneal group by T-test.

Table 4.14: Effects of administration of five therapeutic doses of aqueous plant extracts of *Sonchus asper* at different times on blood glucose levels in alloxan induced diabetic mice

Treatment	Route	Levels of Glucose at Varying Times (mmol/L)					
		0 hour	2 hour	4 hour	6 hour	8 hour	24 hour
Normal control	IP	5.24±0.05	5.26±0.06	5.24±0.04	5.24±0.06	5.28±0.04	5.30±0.03
	Oral	5.16±0.04	5.20±0.04	5.18±0.02	5.22±0.04	5.20±0.03	5.22±0.04
Negative control	IP	15.24±0.78^{kmno}	16.54±0.84^{kmnop}	17.36±0.78^{kmnop}	18.36±0.90^{kmnopq}	19.50±0.82^{mnopq}	21.86±0.75^{opq}
	Oral	14.94±1.17^{JLNQR}	16.48±1.45^{JLNQRS}	17.58±1.54^{JLNQRS}	19.02±1.69^{JLNQRS}	20.82±1.81^{JLNQRS}	22.86±1.65^{LNQRS}
Positive control (insulin)	IP	14.46±0.62	6.56±0.21^{mnoq}	6.06±0.19^{mnopq}	5.38±0.07^{mnop}	5.02±0.07^{mnop}	7.06±0.09^{nq}
Positive control (glibenclamide)	Oral	15.18±1.59^{JL}	11.90±1.80^{JLNQS}	9.02±1.21^{LNQRS}	6.64±0.65^{LNQRS}	5.38±0.22^{NQRS}	8.60±0.70^{LNQRS}
Extract dose (mg/kg body weight)							
25	IP	15.14±0.77	10.98±0.74^{mq}	7.90±0.43^{nopq}	6.78±0.36^{nopq}	5.86±0.33^{nop}	9.20±0.45^{mnoq}
	Oral	15.06±1.06	11.28±0.55^{LNS}	8.60±0.49^{LNQRS}	6.80±0.18^{NQRS}	5.94±0.29^{NQR}	9.18±0.61^{LNQS}
48.4	IP	14.74±0.62	9.44±0.33^{mnq}	7.80±0.25^{mno}	6.52±0.21^{nop}	5.86±0.14^{op}	9.60±0.49^{mq}
	Oral	14.62±1.08	9.82±0.94^{LNQS}	8.90±0.67^{LNQS}	7.08±0.38^{LNQRS}	5.58±.024^{QR}	9.88±0.46^{LNQS}
93.5	IP	13.74±059	9.52±0.44^{mq}	7.44±0.30^{noq}	6.12±0.26^{nop}	5.34±0.13^{op}	9.00±0.49^{mnopq}
	Oral	13.42±0.86	10.50±0.67^{LNS}	8.20±0.53^{LNQS}	6.14±0.47^{NQR}	5.02±1.59^{QR}	9.30±0.48^{LNS}
180.9	IP	13.84±0.60	10.00±0.72^{mq}	7.38±0.34^{noq}	5.92±0.25^{nop}	5.04±0.10^{op}	8.92±0.42^{mnq}
	Oral	13.56±1.11	10.28±0.62^{LNS}	7.92±0.40^{LNQS}	5.96±0.27^{NQR}	5.06±0.06^{QR}	9.04±0.67^{LNS}
350	IP	13.38±0.60	9.40±1.05^{mnq}	7.12±0.85^{mnopq}	5.70±0.37^{nopq}	4.86±0.20^{nopq}	6.54±0.34^{mnopq}
	Oral	13.16±1.14^{JL}	10.32±1.11^{JLNS}	7.60±1.03^{LNQRS}	6.02±0.52^{NQRS}	4.96±0.14^{NQRS}	6.64±0.32^{LNQRS}

Results are expressed as Means ± Standard Deviation for five mice per group. Means accompanied by similar upper case letters and similar lower case letters in the same row are not significantly different at $\rho \leq 0.05$ by ANOVA and post ANOVA (Bonferroni-Holm) test.
Means for IP administration: $^{k}\rho < 0.05$ with respect to 0 hr; $^{m}\rho < 0.05$ with respect to 2 hr; $^{n}\rho < 0.05$ with respect to 4 hr; $^{o}\rho < 0.05$ with respect to 6 hr; $^{p}\rho < 0.05$ with respect to 8 hr; $^{q}\rho < 0.05$ with respect to 24 hr. Means for Oral administration: $^{J}\rho < 0.05$ with respect to 0 hr; $^{L}\rho < 0.05$ with respect to 2 hr; $^{N}\rho < 0.05$ with respect to 4 hr; $^{Q}\rho < 0.05$ with respect to 6 hr; $^{R}\rho < 0.05$ with respect to 8 hr; $^{S}\rho < 0.05$ with respect to 24 hr.

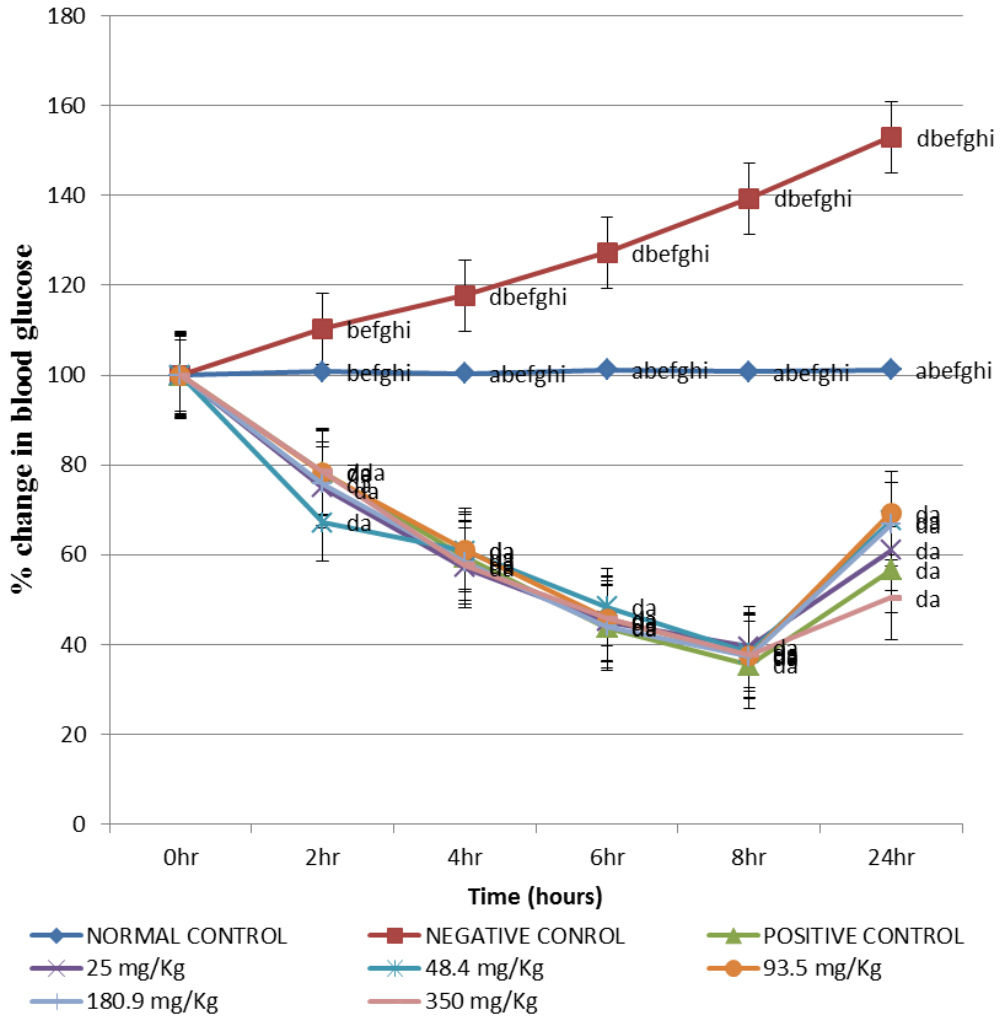

Figure 4.9: Percentage reduction in blood glucose levels at varying times after oral administration of the aqueous extracts of *Sonchus asper* at five different doses in alloxan induced diabetic male mice.

Values are expressed as % means ± SEM for five animals for each time point. $^{d}\rho < 0.05$ when compared to normal control; $^{a}\rho < 0.05$ when compared to negative control; $^{b}\rho < 0.05$ when compared to positive control; $^{e}\rho < 0.05$ when compared to 25 mg/kg body weight; $^{f}\rho < 0.05$ when compared to 48.4 mg/kg body weight; $^{g}\rho < 0.05$ when compared to 93.5 mg/kg body weight; $^{h}\rho < 0.05$ when compared to 180 mg/kg body weight; $^{i}\rho < 0.05$ when compared to 350 mg/kg body weight.

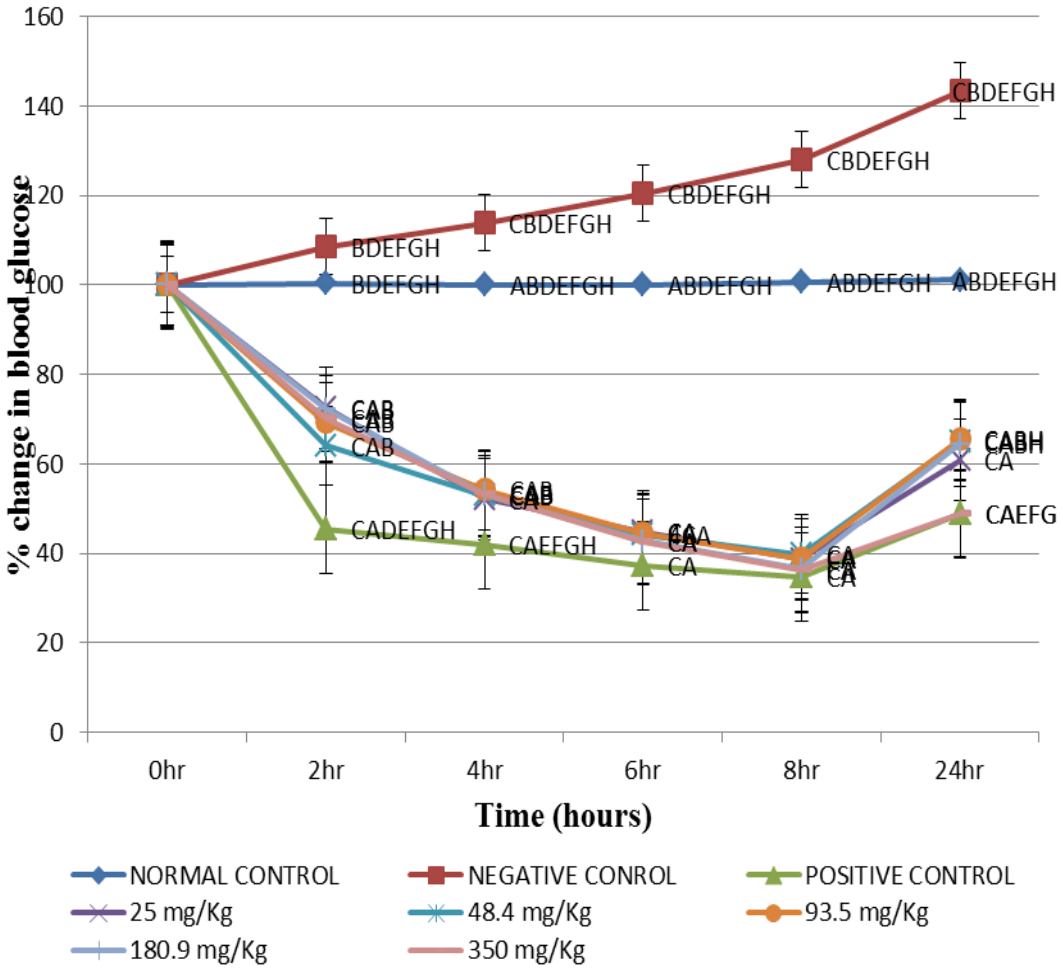

Figure 4.10: Percentage reduction in blood glucose levels at varying times after intraperitoneal administration of the aqueous extracts of *Sonchus asper* at five different doses in alloxan induced diabetic male mice.
Values are expressed as % means ± SEM for five animals for each time point. $^{C}\rho < 0.05$ when compared to normal control; $^{A}\rho < 0.05$ when compared to negative control; $^{B}\rho < 0.05$ when compared to positive control; $^{D}\rho < 0.05$ when compared to 25 mg/kg body weight; $^{E}\rho < 0.05$ when compared to 48.4 mg/kg body weight; $^{F}\rho < 0.05$ when compared to 93.5 mg/kg body weight; $^{G}\rho < 0.05$ when compared to 180 mg/kg body weight; $^{H}\rho < 0.05$ when compared to 350 mg/kg body weight.

Table 4.15 shows the pharmacokinetics of the hypoglycemic activity for the first four hours of the aqueous leaf extracts of *S. asper* of the five tested doses. Results indicate that the pseudo-first order rate constants for the orally administered doses of the aqueous extracts of *S. asper* at 25, 48.4, 93.5, 180.9 and 350 mg/kg body weight doses were 0.2801, 0.2482, 0.474, 0.2689 and 0.2745, respectively, and their accompanying half-lives were 2.47, 2.79, 1.46, 2.58, and 2.52 hours,

respectively. The half-life of 48.4 mg/kg body weight dose is higher while those of the other doses were lower than that of glibenclamide. Pseudo-first order rate constants for the intraperitoneally administered doses of aqueous extracts of *S. asper* at 25, 48.4, 93.5, 180.9 and 350 mg/kg body weight doses were 0.3252, 0.3182, 0.3067, 0.3144 and 0.3154, respectively, and their accompanying half-lives were 2.13, 2.18, 2.26, 2.20 and 2.20 hours, respectively. The half-lives of all the doses were higher than that of insulin. The rate constants for the orally administered aqueous extracts for the five doses ranged from 0.2482 to 0.474 per hour and the half-lives ranged from 2.79 to 1.46 hours, respectively. Rate constants for the intraperitoneally administered aqueous extracts for the five doses ranged from 0.3067 to 0.3252 per hour and the half-lives ranged from 2.26 to 2.13 hours, respectively. The rate constant for insulin was 0.4348 per hour and that of glibenclamide was 0.2603 per hour while their corresponding half-lives were 1.59 and 2.66 hours, respectively.

Table 4.15: Pharmacokinetics of the hypoglycemic activity for the first four hours of the five doses of the aqueous extracts of *Sonchus asper*

Drug (dose)	Route	Rate constant ($hour^{-1}$)	Half-life (hours)
Insulin	IP	0.4348	1.59
Glibenclamide	Oral	0.2603	2.66
Extract (mg/kg bw)			
25	IP	0.3252	2.13
	Oral	0.2801	2.47
48.4	IP	0.3182	2.18
	Oral	0.2482	2.79
93.4	IP	0.3067	2.26
	Oral	0.4740	1.46
180.9	IP	0.3144	2.20
	Oral	0.2689	2.58
350	IP	0.3154	2.20
	Oral	0.2745	2.52

Results are expressed as Means of five mice for each time point; bw represents body weight

4.1.6 Comparison of antidiabetic activity after administration of five aqueous plants extracts at the five doses in mice orally and intraperitoneally

Tables 4.16 and 4.17 shows the hypoglycemic effect of mice orally and intraperitoneally administered with the aqueous extracts at 25 mg/kg body weight of different plants. Results show that for both routes, the hypoglycemic activity of all the plants extracts occurred between the second and the eighth hour where the percentage reduction rate in blood glucose levels are between 57% and 74%. In the second hour, the percentage decrease in blood sugar (orally) were at 85.1% for *B. holstii,* 47.8% for *P. capense,* 74.7% for *V. lasiopus,* 76.2% for *G. parviflora* and 74.9% for *S. asper* (Figure 4.11). By the eighth hour, the blood sugar levels in diabetic mice reduced to 41.5% for *B. holstii,* 30.0% for *P. capense,* 42.8% for *V. lasiopus,* 40.4% for *G. parviflora* and 39.4% for *S. asper* (Figure 4.11).

The mice intraperitoneally administered by the extracts at the same dose showed blood sugar levels lowering from the first to eight hour. By second hour, the extracts had lowered blood sugar levels to 72.7% for *B. holstii,* 43.9% for *P. capense,* 51.9% for *V. lasiopus,* 54.9% for *G. parviflora* and 72.5% for *S. asper* (Figure 4.12). In the eighth hour, the percentage reductions blood glucose levels in diabetic mice were at 40.1% for *B. holstii,* 35.4% for *P. capense,* 29.8% for *V .lasiopus,* 25.8% for *G. parviflora* and 38.7% for *S. asper* (Figure 4.12). *G. parviflora* demonstrated the highest percentage decrease in blood glucose levels at this dose using the intraperitoneal route while *P. capense* showed the highest percentage decrease in blood glucose levels at this dose using the oral route. In the

twenty-fourth hour, mice administered with aqueous extracts at 25 mg/kg body weight dose, had returned to the diabetic states.

Table 4.16: Effects of oral administration of 25 mg/kg body weight of aqueous plants extracts on blood glucose levels at different times in alloxan induced diabetic mice

	0 hour	2 hour	4 hour	6 hour	8 hour	24 hour
B. holstii	15.22±1.77	13.20±1.98^{b}	10.80±1.75^{b}	7.96±1.12^{b}	6.44±0.68^{b}	12.30±1.61
P. capense	13.86±0.28	**6.62±0.78^{a}**	**5.02±0.36^{a}**	**4.5±0.35^{a}**	**4.16±0.35^{a}**	9.82±0.44
V. lasiopus	13.98±1.29	10.44±0.82	7.90±0.55	6.80±0.44	5.98±0.48	9.78±0.65
G. parviflora	13.66±1.06	10.40±1.19	7.64±0.37	6.36±0.41	5.52±0.27	9.56±0.26
S. asper	15.06±1.06	11.28±0.59	8.60±0.49	6.80±0.18	5.94±0.29	9.18±0.61

Results are expressed as Means ± Standard Deviation for five mice per group. Means accompanied by similar lower case letters in the same column are not significantly different at $\rho \leq 0.05$ by ANOVA and post ANOVA (Bonferroni-Holm) test: $^{a}\rho < 0.05$ with respect to *B. holstii*; $^{b}\rho < 0.05$ with respect to *P. capense*; $^{c}\rho < 0.05$ with respect to *V. lasiopus*; $^{d}\rho < 0.05$ with respect to *G. parviflora*; $^{e}\rho < 0.05$ with respect to *S. asper.*

Table 4.17: Effects of intraperitoneal administration of 25 mg/kg body weight of aqueous plants extracts on blood glucose levels at different times in alloxan induced diabetic mice

	0 hour	2 hour	4 hour	6 hour	8 hour	24 hour
B. holstii	14.22±1.03	10.34±0.78^{c}	8.32±0.64^{cd}	6.72±0.54^{cd}	5.70±0.25^{cd}	10.44±0.69
P. capense	14.30±0.69	10.66±0.64	7.72±0.55^{cd}	6.42±0.34^{cd}	5.36±0.25^{cd}	10.70±0.55^{d}
V. lasiopus	13.88±0.47	**7.20±0.58^{ae}**	5.70±0.24^{abe}	**4.48±0.13^{abe}**	**4.14±0.92^{abe}**	9.84±0.38
G. parviflora	13.78±0.39	7.56±0.88	**4.38±0.21^{abe}**	**3.98±0.19^{abe}**	**3.56±0.17^{abe}**	**8.46±0.36^{b}**
S. asper	15.14±0.77	10.98±0.74^{c}	7.90±0.42^{cd}	6.78±0.35^{cd}	5.86±0.33^{cd}	9.20±0.44

Results are expressed as Means ± Standard Deviation for five mice per group. Means accompanied by similar lower case letters in the same column are not significantly different at $\rho \leq 0.05$ by ANOVA and post ANOVA (Bonferroni-Holm) test: $^{a}\rho < 0.05$ with respect to *B. holstii*; $^{b}\rho < 0.05$ with respect to *P. capense*; $^{c}\rho < 0.05$ with respect to *V. lasiopus*; $^{d}\rho < 0.05$ with respect to *G. parviflora*; $^{e}\rho < 0.05$ with respect to *S. asper*

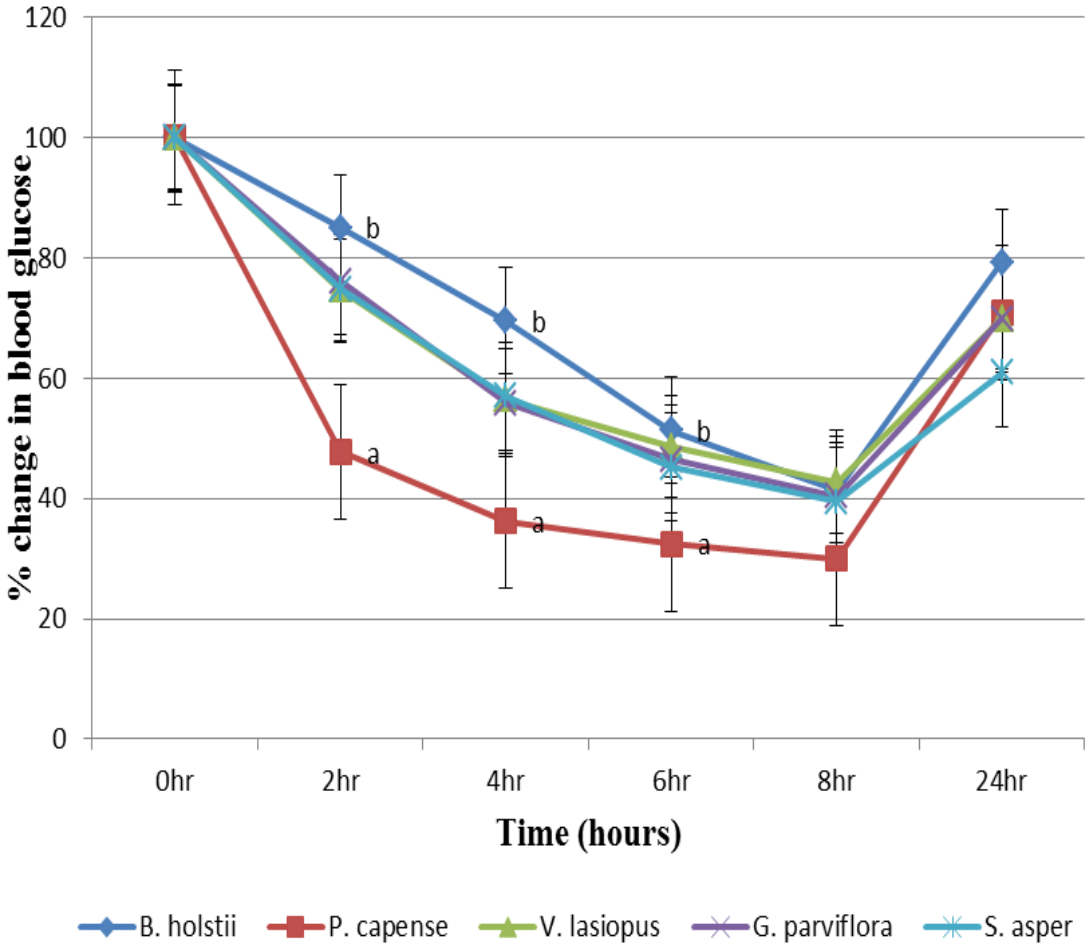

Figure 4.11: Percentage reduction in blood sugar levels in alloxan induced diabetic mice orally administered with aqueous extracts at 25 mg/kg body weight.
Values are expressed as % means ± SEM for five animals for each time point. $^{a}\rho < 0.05$ when compared to *B. holstii*, $^{b}\rho < 0.05$ when compared to *P. capense*, $^{c}\rho < 0.05$ when compared to *V. lasiopus*, $^{d}\rho < 0.05$ when compared to *G. parviflora*, $^{e}\rho < 0.05$ when compared to *S.asper*.

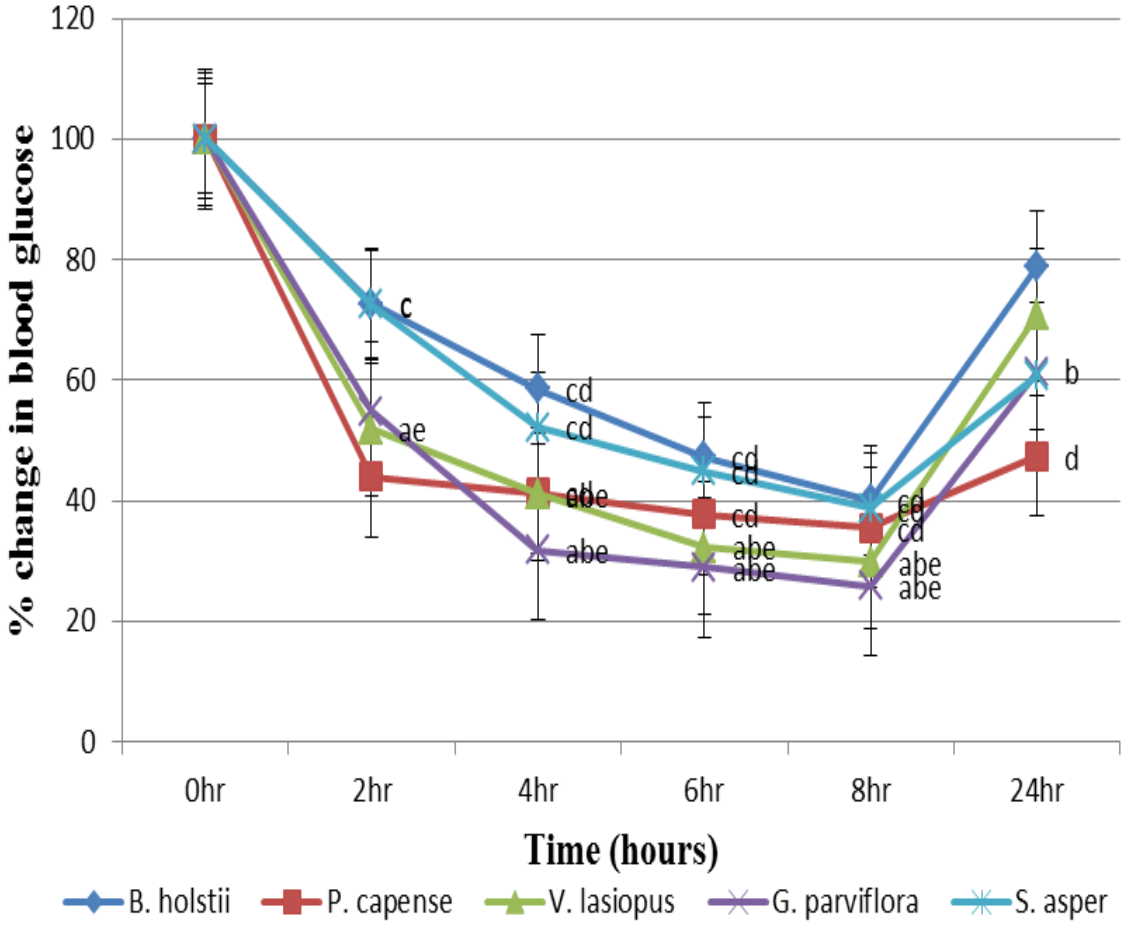

Figure 4.12: Percentage reduction in blood sugar levels in alloxan induced diabetic mice intraperitoneally administered with aqueous extracts at 25 mg/kg body weight.
Values are expressed as % means ± SEM for five animals for each time point. [a]ρ < 0.05 when compared to *B. holstii*, [b]ρ < 0.05 when compared to *P. capense*, [c]ρ < 0.05 when compared to *V. lasiopus*, [d]ρ < 0.05 when compared to *G. parviflora*, [e]ρ < 0.05 when compared to *S.asper*.

Tables 4.18 and 4.19 show the hypoglycemic effect of mice intraperitoneally and orally administered with 48.4 mg/kg body weight of different aqueous plants extracts. Results show that for both routes, the hypoglycemic activity for all the five plants extracts occurred between the second and the eighth hour where the percentage reduction rate in blood sugar are between 20.9% and 67.2%. In the second hour, the percentage reductions in blood sugar level (orally) in diabetic mice were 66.3% for *B. holstii*, 57.2% for *P. capense*, 74.7% for *V. lasiopus*,

76.1% for *G. parviflora* and 74.9% for *S. asper* (Figure 4.13). By the eight hour, the blood sugar levels in diabetic mice reduced to 30.0% for *B. holstii,* 27.3% for *P. capense,* 42.8**%** for *V. lasiopus,* 40.4% for *G. parviflora* and 39.4**%** for *S. asper* (Figure 4.13).

The mice intraperitoneally administered by the extracts at the same dose showed blood sugar levels lowering from hour one to hour eight. By second hour, the five aqueous extracts lowered blood sugar levels in diabetic mice to 63.4% for *B. holstii,* 55.7% for *P. capense,* 39.3% for *V. lasiopus,* 43.7% for *G. parviflora* and 64.0% for *S. asper* (Figure 4.14). In the eighth hour, the percentage reductions in blood glucose levels in diabetic mice were 31.5% for *B. holstii,* 28.1% for *P. capense,* 20.9% for *V. lasiopus,* 24.7% for *G. parviflora* and 39.8% for *S. asper* (Figure 4.14). *P. capense* demonstrated the highest percentage reduction in blood sugar levels in diabetic mice at this dose via the oral route while *V. lasiopus* and *G. parviflora* showed the highest percentage in blood glucose lowering via the intraperitoneal route. In the twenty-fourth hour, diabetic mice administered with aqueous plants extracts at 48.4 mg/kg body weight dose had returned to the diabetic states.

Table 4.18: Effects of oral administration of 48.4 mg/kg body weight of aqueous plants extracts of on blood glucose levels at different times in alloxan induced diabetic mice

	0 hour	2 hour	4 hour	6 hour	8 hour	24 hour
B. holstii	15.60±2.27	10.34±1.39	6.80±0.61	5.58±0.47^{b}	4.68±0.27^{b}	10.12±1.07
P. capense	11.88±1.85	6.80±0.73	**5.16±0.62^{e}**	**3.96±0.45^{ae}**	**3.24±0.31^{ae}**	9.50±0.44
V. lasiopus	14.44±0.43	9.08±0.43	**6.50±0.39^{e}**	**4.70±0.21^{e}**	**4.04±0.26^{e}**	8.20±0.39
G. parviflora	14.48±0.70	9.48±0.47	**6.46±0.40^{e}**	**4.74±0.20^{e}**	**3.92±0.32^{e}**	8.42±0.46
S. asper	14.62±1.68	9.82±0.93	8.96±0.67bcd	7.08±0.38^{bcd}	5.58±0.24^{bcd}	9.88±0.46

Results are expressed as Means ± Standard Deviation for five mice per group. Means accompanied by similar lower case letters in the same column are not significantly different at $\rho \leq 0.05$ by ANOVA and post ANOVA (Bonferroni-Holm) test: $^{a}\rho < 0.05$ with respect to *B. holstii*; $^{b}\rho < 0.05$ with respect to *P. capense*; $^{c}\rho < 0.05$ with respect to *V. lasiopus*; $^{d}\rho < 0.05$ with respect to *G. parviflora*; $^{e}\rho < 0.05$ with respect to *S. asper.*

Table 4.19: Effects of intraperitoneal administration of 48.4 mg/kg body weight of aqueous plants extracts on blood glucose levels at different times in alloxan induced diabetic mice

	0 hour	2 hour	4 hour	6 hour	8 hour	24 hour
B. holstii	15.42±1.33	9.78±1.17^{cd}	6.92±0.70^{cd}	5.56±0.28^{cd}	4.86±0.18^{cd}	9.34±0.90
P. capense	14.58±0.67	8.12±0.45	6.22±0.46^{d}	5.08±0.32^{cde}	4.10±0.44^{ce}	9.62±0.46
V. lasiopus	14.24±0.84	**5.60±0.47^{ae}**	4.54±0.46^{ae}	**3.36±0.25^{abe}**	**2.98±0.22^{abe}**	8.02±0.12
G. parviflora	13.22±0.93	**5.78±0.62^{ae}**	**4.14±0.25^{abe}**	**3.62±0.21^{abe}**	**3.26±0.20^{ae}**	7.94±0.50
S. asper	14.74±1.39	9.44±0.33^{cd}	7.80±0.24^{cd}	6.25±0.20^{bcd}	5.86±0.14^{bcd}	9.60±0.49

Results are expressed as Means ± Standard Deviation for five mice per group. Means accompanied by similar lower case letters in the same column are not significantly different at $\rho \leq 0.05$ by ANOVA and post ANOVA (Bonferroni-Holm) test: $^{a}\rho < 0.05$ with respect to *B. holstii*; $^{b}\rho < 0.05$ with respect to *P. capense*; $^{c}\rho < 0.05$ with respect to *V. lasiopus*; $^{d}\rho < 0.05$ with respect to *G. parviflora*; $^{e}\rho < 0.05$ with respect to *S. asper.*

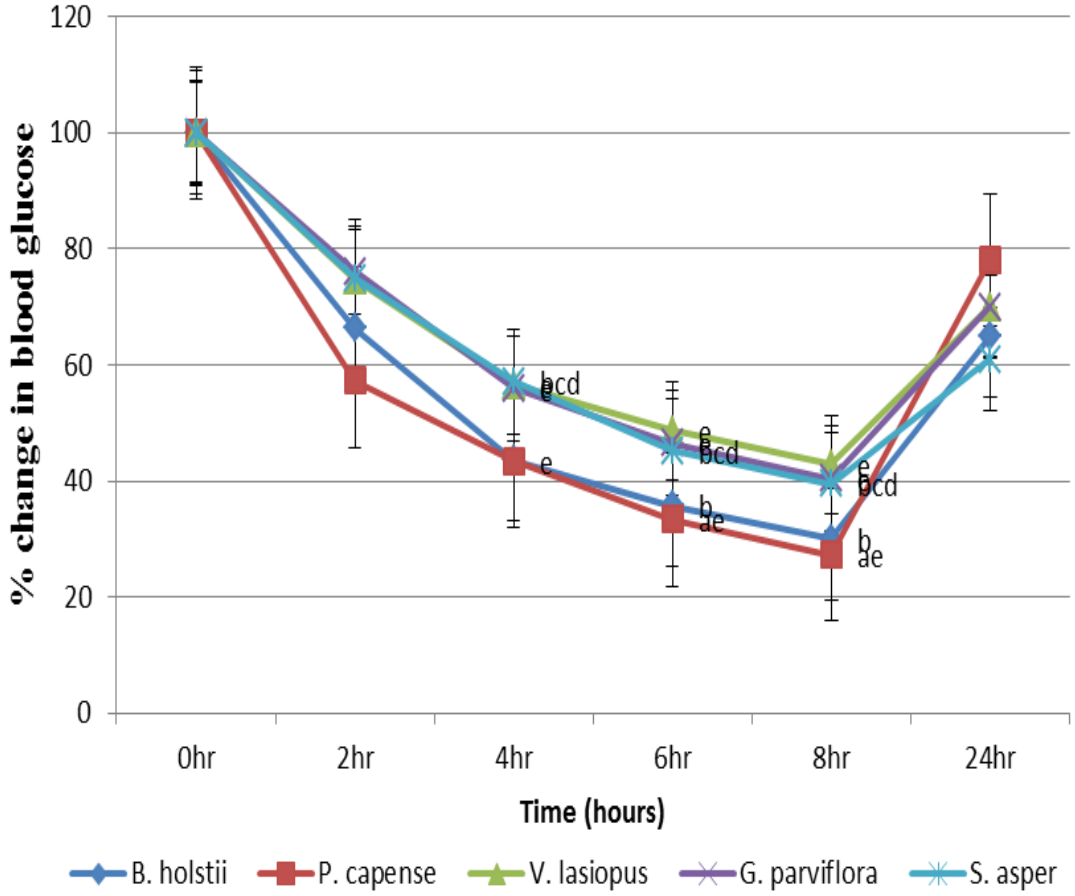

Figure 4.13: Percentage reduction in blood sugar levels in alloxan induced diabetic mice orally administered with aqueous plants extracts at 48.4mg/kg body weight
Values are expressed as % means ± SEM for five animals for each time point. $^{a}\rho < 0.05$ when compared to *B. holstii*, $^{b}\rho < 0.05$ when compared to *P. capense*, $^{c}\rho < 0.05$ when compared to *V. lasiopus*, $^{d}\rho < 0.05$ when compared to *G. parviflora*, $^{e}\rho < 0.05$ when compared to *S.asper*.

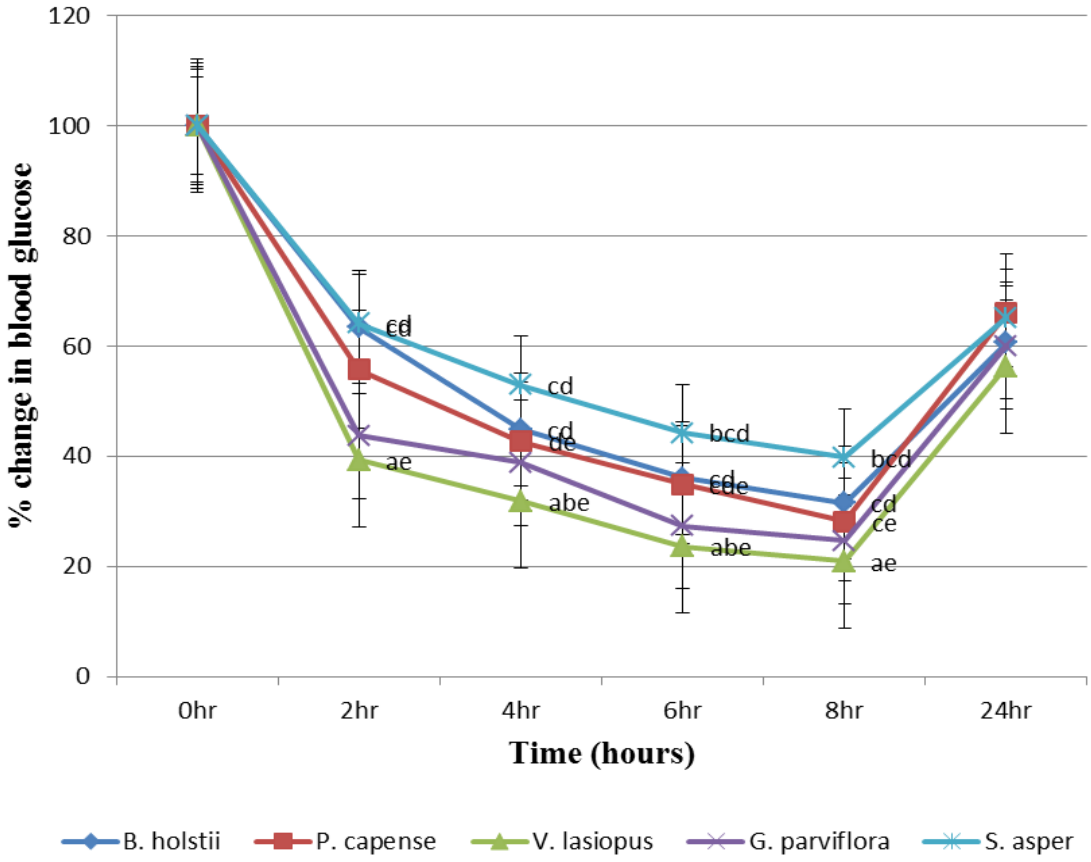

Figure 4.14: Percentage reduction in blood sugar levels in alloxan induced diabetic mice intraperitoneally administered with aqueous plants extracts at 48.4mg/kg body weight.
Values are expressed as % means ± SEM for five animals for each time point. [a]ρ < 0.05 when compared to *B. holstii*, [b]ρ < 0.05 when compared to *P. capense*, [c]ρ < 0.05 when compared to *V. lasiopus*, [d]ρ < 0.05 when compared to *G. parviflora*, [e]ρ < 0.05 when compared to *S.asper*.

Tables 4.20 and 4.21 show the hypoglycemic effect of mice intraperitoneally and orally administered with 93.5 mg/kg body weight of different plants extracts. Results show that in both routes, the hypoglycemic activity for all the five aqueous extracts occurred between the second and the eighth hour where the percentage reduction rate in blood sugar are between 23.5% and 78.7%. In the second hour, the percentage reductions in blood sugar level (orally) in diabetic mice were 65.2% for *B. holstii*, 58.7% for *P. capense*, 67.2% for *V. lasiopus*, 68.9% for *G. parviflora*

and 78.2% for *S. asper* (Figure 4.15). By the eighth hour, the blood sugar levels in diabetic mice reduced to 33.9% for *B. holstii,* 27.5% for *P. capense*, 25.3% for *V. lasiopus*, 25.4% for *G. parviflora* and 37.4% for *S. asper* (Figure 4.15).

The mice intraperitoneally administered with the five aqueous extracts at the same dose showed blood sugar levels lowering from hour two to hour eight. By second hour, the five aqueous extracts lowered blood sugar levels in diabetic mice to 57.5% for *B. holstii,* 55.5% for *P. capense*, 44.1% for *V. lasiopus*, 47.5% for *G. parviflora* and 69.3% for *S. asper* (Figure 4.16). In the eighth hour, the percentage reductions in blood glucose levels in diabetic mice by the five aqueous extracts were 30.6% for *B. holstii*, 23.3% for *P. capense*, 26.4% for *V. lasiopus,* 27.5% for *G. parviflora* and 38.9% for *S. asper* (Figure 4.16). *P. capense* demonstrated the highest blood glucose reduction in diabetic mice at this dose via the oral route while *V. lasiopus* and *G. parviflora* demonstrated the highest blood sugar reduction via the intraperitoneal route. In the twenty-fourth hour, diabetic mice administered with aqueous extracts at 93.5 mg/kg body weight dose had returned to the diabetic states.

Table 4.20: Effects of oral administration of 93.5 mg/kg body weight of aqueous extracts of on blood glucose levels at different times in alloxan induced diabetic mice

	0 hour	2 hour	4 hour	6 hour	8 hour	24 hour
B. holstii	14.32±1.38	9.34±1.44	6.86±0.93	5.58±0.56	4.86±0.46d	10.32±0.87d
P. capense	13.38±0.37	7.86±0.49	5.44±0.53	**4.40±0.34e**	**3.68±0.34e**	8.38±0.35
V. lasiopus	14.44±0.63	9.70±0.58	6.74±0.72	4.48±0.37	**3.66±0.20e**	7.90±0.35
G. parviflora	13.44±0.65	9.26±0.34	6.86±0.58	4.54±0.18	**3.42±0.24ae**	7.52±0.37a
S. asper	13.42±0.25	10.56±0.66	8.20±0.53	6.14±0.46b	5.02±0.15bcd	9.30±0.48

Results are expressed as Means ± Standard Deviation for five mice per group. Means accompanied by similar lower case letters in the same column are not significantly different at $\rho \leq 0.05$ by ANOVA and post ANOVA (Bonferroni-Holm) test: $^{a}\rho < 0.05$ with respect to *B. holstii*; $^{b}\rho < 0.05$ with respect to *P. capense*; $^{c}\rho < 0.05$ with respect to *V. lasiopus*; $^{d}\rho < 0.05$ with respect to *G. parviflora*; $^{e}\rho < 0.05$ with respect to *S. asper.*

Table 4.21: Effects of intraperitoneal administration of 93.5 mg/kg body weight of aqueous extracts of on blood glucose levels at different times in alloxan induced diabetic mice

	0 hour	2 hour	4 hour	6 hour	8 hour	24 hour
B. holstii	14.46±0.81	8.32±0.70	6.06±0.42	4.96±0.09	4.42±4.01bde	9.34±0.49^{cd}
P. capense	14.42±0.46	8.00±0.14	**5.46±0.42^{e}**	**4.40±0.40^{e}**	**3.36±0.19^{ae}**	8.68±0.56
V. lasiopus	14.10±0.23	**6.22±0.28^{e}**	**5.26±0.26^{e}**	**4.30±0.31^{e}**	**3.72±0.18^{e}**	**7.30±0.28^{a}**
G. parviflora	12.88±0.65	**6.12±0.76^{e}**	**4.68±0.64^{e}**	**4.18±0.44^{e}**	**3.54±0.31^{ae}**	**7.32±0.29^{a}**
S. asper	13.74±0.59	9.52±0.43^{cd}	7.44±0.30^{bcd}	6.12±0.26^{bcd}	5.34±0.13^{abcd}	9.00±0.48^{c}

Results are expressed as Means ± Standard Deviation for five mice per group. Means accompanied by similar lower case letters in the same column are not significantly different at $\rho \leq 0.05$ by ANOVA and post ANOVA (Bonferroni-Holm) test: $^{a}\rho < 0.05$ with respect to *B. holstii*; $^{b}\rho < 0.05$ with respect to *P. capense*; $^{c}\rho < 0.05$ with respect to *V. lasiopus*; $^{d}\rho < 0.05$ with respect to *G. parviflora*; $^{e}\rho < 0.05$ with respect to *S. asper.*

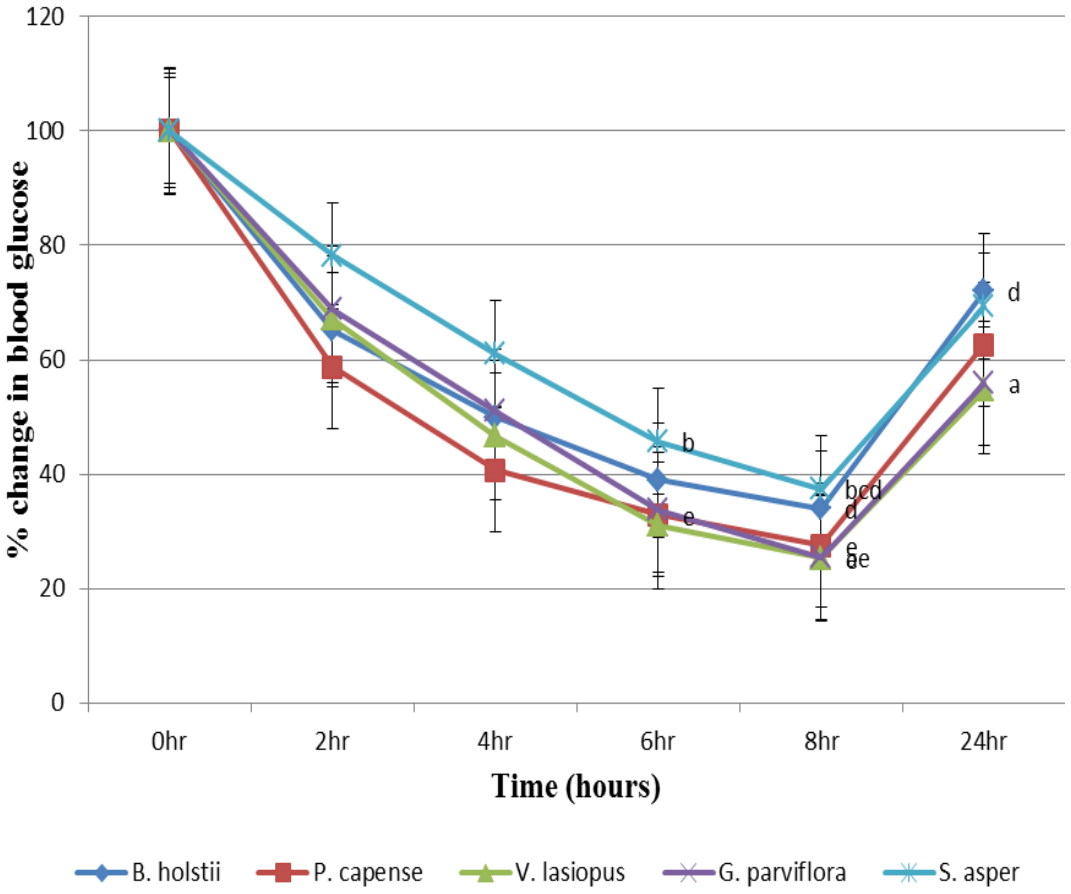

Figure 4.15: Percentage reduction in blood sugar levels in alloxan induced diabetic mice orally administered with aqueous extracts at 93.5 mg/kg body weight
Values are expressed as % means ± SEM for five animals for each time point. $^{a}\rho < 0.05$ when compared to *B. holstii*, $^{b}\rho < 0.05$ when compared to *P. capense*, $^{c}\rho < 0.05$ when compared to *V. lasiopus*, $^{d}\rho < 0.05$ when compared to *G. parviflora*, $^{e}\rho < 0.05$ when compared to *S.asper*.

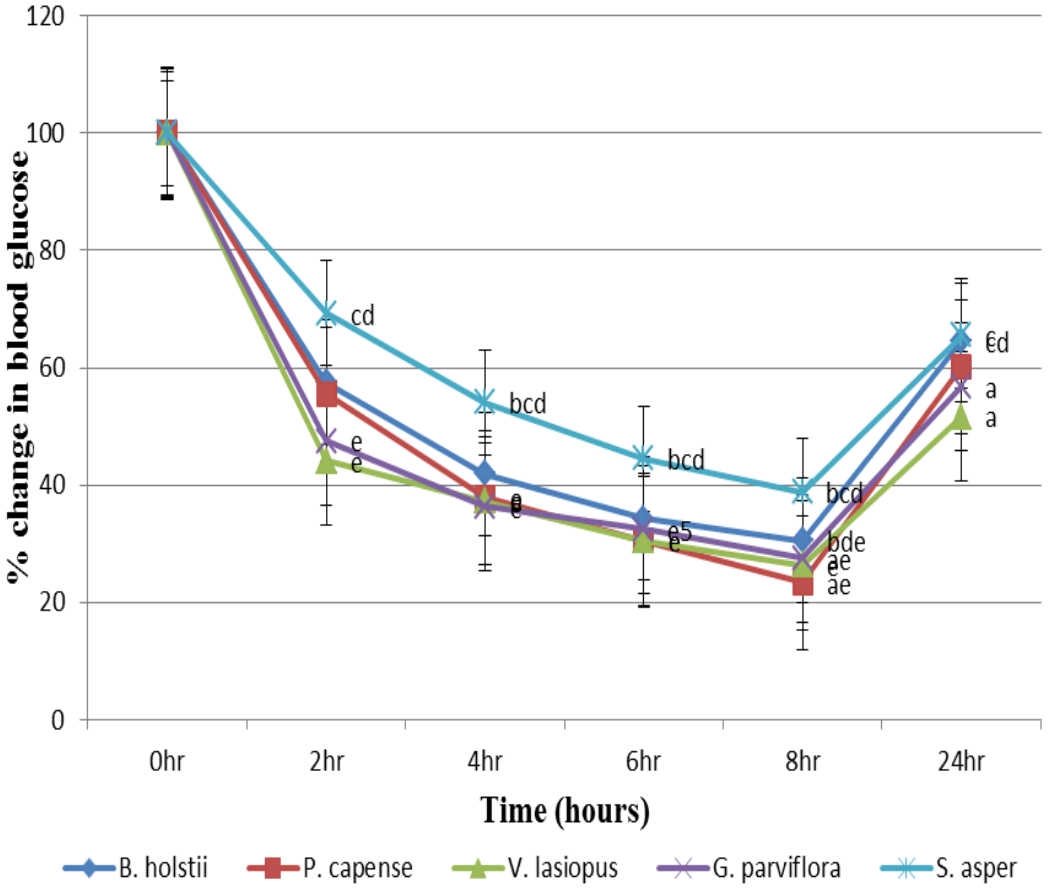

Figure 4.16: Percentage reduction in blood sugar levels in alloxan induced diabetic mice intraperitoneally administered with aqueous extracts at 93.5 mg/kg body weight.
Values are expressed as % means ± SEM for five animals for each time point. [a]ρ < 0.05 when compared to *B. holstii*, [b]ρ < 0.05 when compared to *P. capense*, [c]ρ < 0.05 when compared to *V. lasiopus*, [d]ρ < 0.05 when compared to *G. parviflora*, [e]ρ < 0.05 when compared to *S.asper*.

Tables 4.22 and 4.23 show the hypoglycemic effect of mice intraperitoneally and orally administered with 180.9mg/kg body weight of different plant extracts. Results show that in both routes, the hypoglycemic activity for all the extracts occurred between the second and the eighth hour where the percentage reduction rate in blood sugar are between 21.9% and 72.3%. In the second hour, the decrease in blood sugar (orally) in diabetic mice were 73.7% for *B. holstii*, 57.5% for *P. capense*, 59.9% for *V .lasiopus*, 61.3% for *G. parviflora* and 75.8% for *S. asper*

(Figure 4.17). By the eighth hour, the blood sugar levels in diabetic mice reduced to 38.7% for *B. holstii,* 32.9% for *P. capense,* 30.9% for *V. lasiopus,* 25.0% for *G. parviflora* and 37.3% for *S. asper* (Figure 4.17).

Mice intraperitoneally administered with the five aqueous extracts at 180.9mg/kg body weight showed blood sugar lowering from hour two to hour eight. By the second hour, the five aqueous extracts at 180.9mg/kg body weight lowered blood sugar levels in diabetic mice to 67.6% for *B. holstii,* 51.7% for *P. capense,* 49.2% for *V. lasiopus,* 52.0% for *G. parviflora* and 72.2 % for *S. asper* (Figure 4.18). In the eighth hour, the five aqueous extracts at 180.9mg/kg body weight lowered blood glucose to 34.5% for *B. holstii,* 24.6% for *P. capense,* 21.9% for *V. lasiopus,* 31.0% for *G. parviflora* and 36.4% for *S. asper* (Figure 4.18). *G. parviflora* demonstrated the highest blood glucose lowering in diabetic mice at this dose via the oral route while *V. lasiopus* demonstrated the highest blood sugar lowering in diabetic mice via the intraperitoneal route. In the twenty-fourth hour, diabetic mice administered with aqueous extracts at 180.9 mg/kg body weight dose had returned to the diabetic states.

Table 4.22: Effects of oral administration of 180.9 mg/kg body weight of aqueous extracts on blood glucose levels at different times in alloxan induced diabetic mice

	0 hour	2 hour	4 hour	6 hour	8 hour	24 hour
B. holstii	14.22±1.62	10.48±1.20[b]	7.90±0.75	5.72±0.54	5.50±0.68d	9.90±0.89[d]
P. capense	12.60±0.72	7.24±0.66a[e]	5.56±0.59	4.78±0.59	4.14±0.38	8.40±0.80
V. lasiopus	14.78±0.82	8.86±0.45	7.12±0.36	5.72±0.42	4.56±0.44	7.16±0.21
G. parviflora	14.26±1.26	8.74±0.56	6.22±0.65	4.54±0.46	**3.56±0.25**[a]	**6.96±0.48**[a]
S. asper	13.56±1.10	10.28±0.50[b]	7.92±0.40	5.96±0.26	5.06±0.06	9.04±0.67

Results are expressed as Means ± Standard Deviation for five mice per group. Means accompanied by similar lower case letters in the same column are not significantly different at $\rho \leq 0.05$ by ANOVA and post ANOVA (Bonferroni-Holm) test: [a]$\rho < 0.05$ with respect to *B. holstii*; [b]$\rho < 0.05$ with respect to *P. capense*; [c]$\rho < 0.05$ with respect to *V. lasiopus*; [d]$\rho < 0.05$ with respect to *G. parviflora*; [e]$\rho < 0.05$ with respect to *S. asper*

Table 4.23: Effects of intraperitoneal administration of 180.9 mg/kg body weight of aqueous extracts on blood glucose levels at different times in alloxan induced diabetic mice

	0 hour	2 hour	4 hour	6 hour	8 hour	24 hour
B. holstii	13.7±1.28	9.26±0.76	7.36±0.62bc	5.48±0.50c	4.72±0.40c	9.48±0.85[cd]
P. capense	14.16±0.61	7.32±0.57	**5.16±0.33**[ae]	**4.18±0.31**[e]	**3.48±0.20**[e]	8.54±0.63
V. lasiopus	13.16±0.68	**6.48±0.70**[e]	**4.74±0.62**[ae]	**3.44±0.29**[ae]	**2.88±0.32**[ae]	**6.94±0.19**[a]
G. parviflora	13.34±0.43	**6.94±0.75**[e]	5.60±0.36	4.72±0.35	4.14±0.39	**6.76±0.48**[a]
S. asper	13.84±1.33	10.00±0.72[cd]	7.38±0.34[bc]	5.92±0.24[bc]	5.04±0.10	8.92±0.41

Results are expressed as Means ± Standard Deviation for five mice per group. Means accompanied by similar lower case letters in the same column are not significantly different at $\rho \leq 0.05$ by ANOVA and post ANOVA (Bonferroni-Holm) test: [a]$\rho < 0.05$ with respect to *B. holstii*; [b]$\rho < 0.05$ with respect to *P. capense*; [c]$\rho < 0.05$ with respect to *V. lasiopus*; [d]$\rho < 0.05$ with respect to *G. parviflora*; [e]$\rho < 0.05$ with respect to *S. asper.*

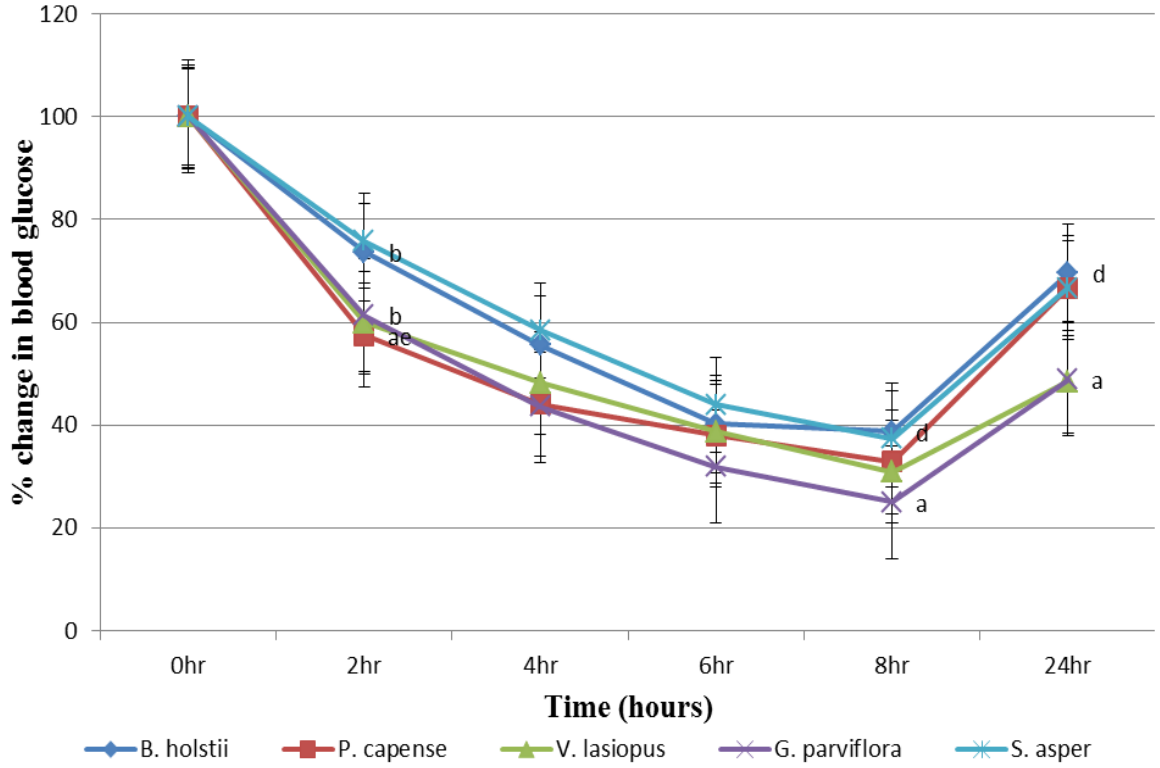

Figure 4.17: Percentage reduction in blood sugar levels in alloxan induced diabetic mice orally administered with aqueous extracts at 180.9 mg/kg body weight
Values are expressed as % means ± SEM for five animals for each time point. $^{a}\rho < 0.05$ when compared to *B. holstii*, $^{b}\rho < 0.05$ when compared to *P. capense*, $^{c}\rho < 0.05$ when compared to *V. lasiopus*, $^{d}\rho < 0.05$ when compared to *G. parviflora*, $^{e}\rho < 0.05$ when compared to *S.asper*.

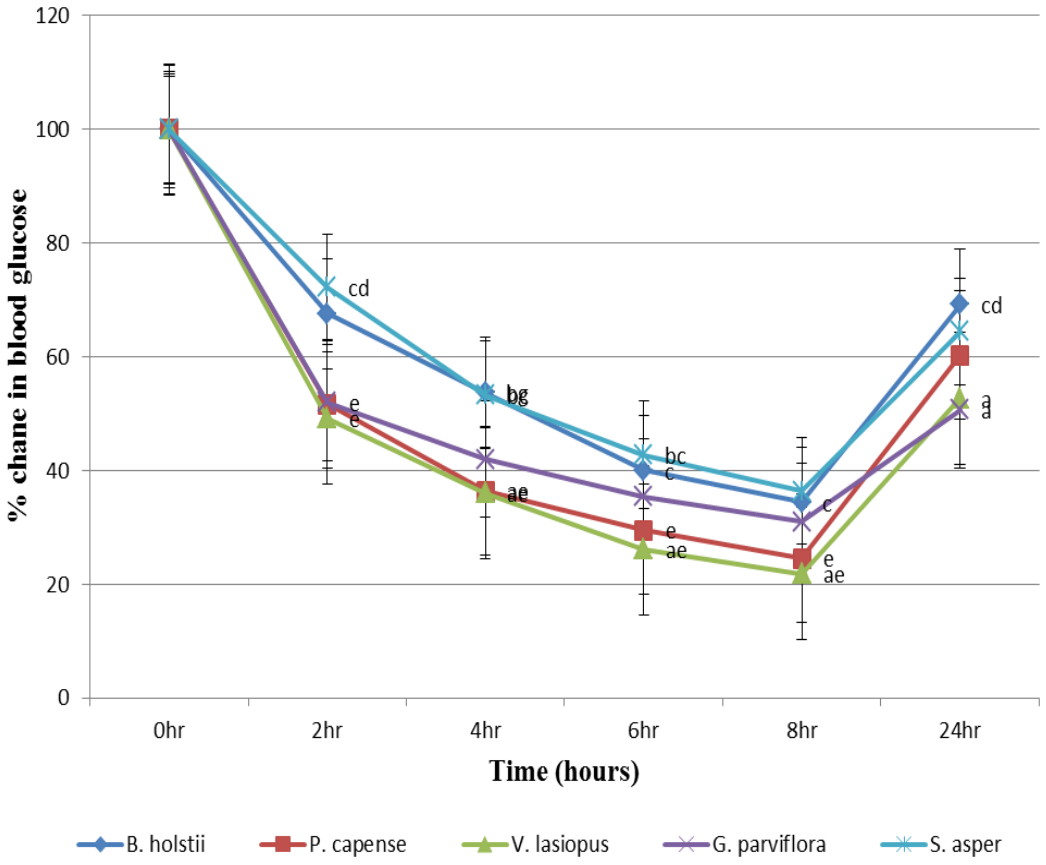

Figure 4.18: Percentage reduction in blood sugar levels in alloxan induced diabetic mice intraperitoneally administered with aqueous extracts at 180.9 mg/kg body weight.
Values are expressed as % means ± SEM for five animals for each time point. $^{a}\rho < 0.05$ when compared to *B. holstii*, $^{b}\rho < 0.05$ when compared to *P. capense*, $^{c}\rho < 0.05$ when compared to *V. lasiopus*, $^{d}\rho < 0.05$ when compared to *G. parviflora*, $^{e}\rho < 0.05$ when compared to *S.asper*.

Tables 4.24 and 4.25 show the hypoglycemic effect of intraperitoneal and oral administration of 350 mg/kg body weight of different plant extracts in diabetic mice. Results show that in both routes, the hypoglycemic activity for all the extracts occurred between the second and the eighth hour where the reduction in blood sugar are between 18.6% and 78.4%. In the second hour, the percentage decrease in blood sugar (orally) in diabetic mice were 77.5% for *B. holstii*, 47.4% for *P. capense*, 69.7% for *V. lasiopus*, 72.6% for *G. parviflora* and 78.4% for *S. asper* (Figure 4.19). By the eighth hour, the five aqueous extracts at 350mg/kg

body weight lowered blood sugar levels in diabetic mice to 33.9% for *B. holstii*, 21.7% for *P. capense*, 29.6% for *V. lasiopus*, 31.9% for *G. parviflora* and 37.7% for *S. asper* (Figure 4.19).

Diabetic mice intraperitoneally administered with the five aqueous extracts at 350mg/kg body weight dose showed blood sugar levels lowering from hour two to hour eight. By the second hour, the aqueous extracts at 350mg/kg body weight lowered blood sugar levels to 73.2% for *B. holstii*, 48.6% for *P. capense*, 52.4% for *V. lasiopus*, 47.1% for *G. parviflora* and 70.3% for *S. asper* (Figure 4.20). In the eighth hour, the five aqueous extracts at 350mg/kg body weight reduced blood glucose levels to 33.2% for *B. holstii*, 18.6% for *P. capense*, 23.7% for *V. lasiopus*, 23.5% for *G. parviflora* and 36.3% for *S. asper* (Figure 4.20). *P. capense* extracts at 350mg/kg body weight demonstrated the highest decrease in blood glucose levels in diabetic mice via the oral and intraperitoneal route. In the twenty-fourth hour, diabetic mice administered with aqueous extracts at 350 mg/kg body weight dose had not returned to the diabetic states.

Table 4.24: Effects of oral administration of 350 mg/kg body weight of aqueous extracts on blood glucose levels at different times in alloxan induced diabetic mice

	0 hour	2 hour	4 hour	6 hour	8 hour	24 hour
B. holstii	16.36±1.70	12.68±1.70^{b}	9.78±1.45^{b}	7.66±0.83^{b}	5.54±0.47^{bc}	7.24±0.42^{cd}
P. capense	12.28±0.54	**5.82±1.07^{a}**	**3.70±0.23^{acde}**	**3.16±0.27^{acde}**	**2.66±0.26^{acd}**	7.10±0.57^{cd}
V. lasiopus	14.60±0.86	10.18±1.03	7.48±0.22^{b}	5.52±0.28^{b}	4.32±0.16^{ab}	5.14±0.40^{ab}
G. parviflora	13.92±0.83	10.10±0.56	8.18±0.42^{b}	5.58±0.39^{b}	4.44±0.28^{b}	**4.58±0.35^{be}**
S. asper	13.16±1.14	10.32±1.10	7.60±1.03^{b}	6.02±1.15^{b}	4.96±0.14^{b}	6.64±0.31^{d}

Results are expressed as Means ± Standard Deviation for five mice per group. Means accompanied by similar lower case letters in the same column are not significantly different at $\rho \leq 0.05$ by ANOVA and post ANOVA (Bonferroni-Holm) test: $^{a}\rho < 0.05$ with respect to *B. holstii*; $^{b}\rho < 0.05$ with respect to *P. capense*; $^{c}\rho < 0.05$ with respect to *V. lasiopus*; $^{d}\rho < 0.05$ with respect to *G. parviflora*; $^{e}\rho < 0.05$ with respect to *S. asper.*

Table 4.25: Effects of intraperitoneal administration of 350 mg/kg body weight of aqueous extracts on blood glucose levels at different times in alloxan induced diabetic mice

	0 hour	2 hour	4 hour	6 hour	8 hour	24 hour
B. holstii	15.5±1.37	11.36±1.40^{bcd}	8.30±0.66^{bcd}	6.74±0.38^{bcd}	5.14±0.11^{bcd}	7.14±0.24^{d}
P. capense	13.78±0.74	**6.70±0.52^{a}**	**4.38±0.19^{ae}**	**3.46±0.28^{ae}**	**2.56±0.13^{ae}**	5.54±0.38
V. lasiopus	12.66±0.71	**6.64±0.73^{a}**	**4.66±0.62^{a}**	**3.48±0.42^{ae}**	**3.00±0.42^{ae}**	5.58±0.59
G. parviflora	13.26±0.49	**6.24±0.72^{a}**	**4.66±0.34^{a}**	**3.86±0.28^{ae}**	**3.12±0.13^{ae}**	**5.30±0.30^{a}**
S. asper	13.38±0.59	9.40±1.05	7.12±0.84^{b}	5.70±0.37^{bcd}	4.86±0.20^{bcd}	6.54±0.34

Results are expressed as Means ± Standard Deviation for five mice per group. Means accompanied by similar lower case letters in the same column are not significantly different at $\rho \leq 0.05$ by ANOVA and post ANOVA (Bonferroni-Holm) test: $^{a}\rho < 0.05$ with respect to *B. holstii*; $^{b}\rho < 0.05$ with respect to *P. capense*; $^{c}\rho < 0.05$ with respect to *V. lasiopus*; $^{d}\rho < 0.05$ with respect to *G. parviflora*; $^{e}\rho < 0.05$ with respect to *S. asper.*

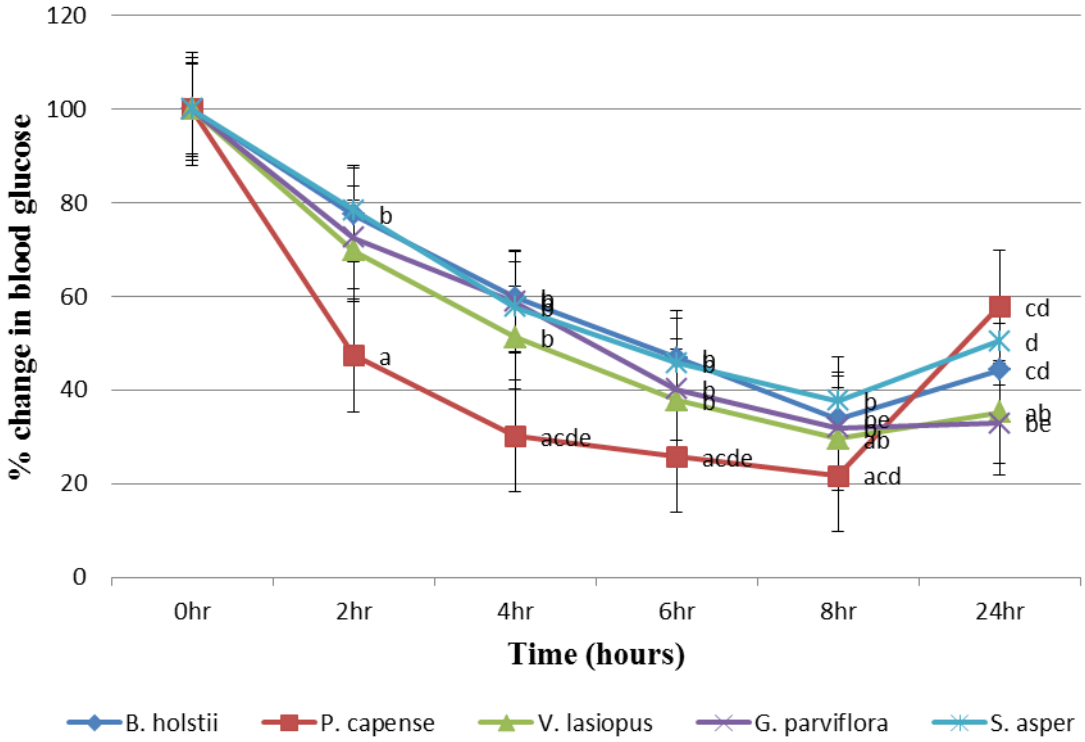

Figure 4.19: Percentage reduction in blood sugar levels in alloxan induced diabetic mice orally administered with aqueous extracts at 350 mg/kg body weight

Values are expressed as % means ± SEM for five animals for each time point. [a]ρ < 0.05 when compared to *B. holstii*, [b]ρ < 0.05 when compared to *P. capense*, [c]ρ < 0.05 when compared to *V. lasiopus*, [d]ρ < 0.05 when compared to *G. parviflora*, [e]ρ < 0.05 when compared to *S.asper*.

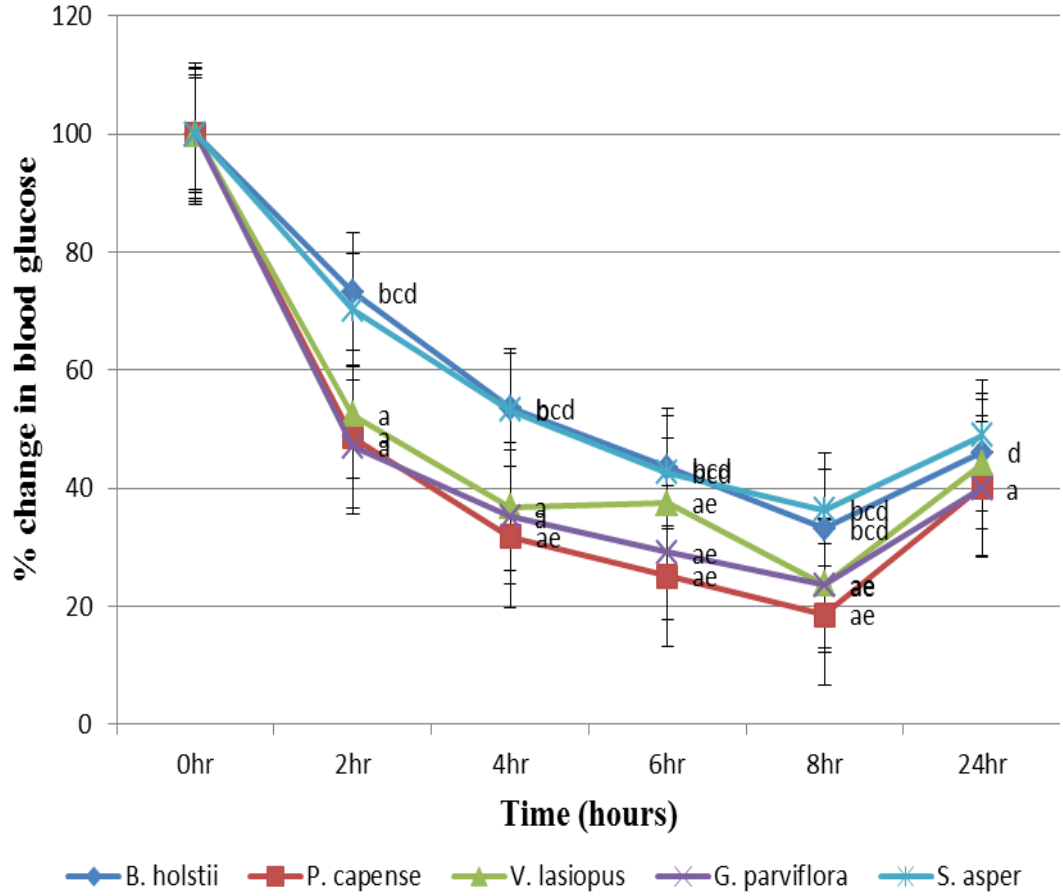

Figure 4.20: Percentage reduction in blood sugar levels in alloxan induced diabetic mice intraperitoneally administered with aqueous extracts at 350 mg/kg body weight.

Values are expressed as % means ± SEM for five animals for each time point. [a]ρ < 0.05 when compared to *B. holstii*, [b]ρ < 0.05 when compared to *P. capense*, [c]ρ < 0.05 when compared to *V. lasiopus*, [d]ρ < 0.05 when compared to *G. parviflora*, [e]ρ < 0.05 when compared to *S.asper*.

4.2 *In Vivo* Safety of the Aqueous Extracts of *B. holstii, P. capense, V. lasiopus, G. parviflora* and *S. asper* in normal mice

4.2.1 Effect of oral administration of aqueous extracts of *B. holstii, P. capense, V. lasiopus, G. parviflora* and *S. asper* on body weight and mean weekly body weight change

As depicted in Table 4.26, the daily oral administration of aqueous extracts of *B. holstii, P. capense, V. lasiopus,* and *G. parviflora* at dose levels of 450, 670 and 1000 mg/kg body weight daily for 28 days significantly reduced the body weight and mean weekly body weight gain in a dose dependent manner compared to the normal control mice. Mice treated with the aqueous extracts of *S. asper* significantly reduced the body weight and mean weekly body weight gain at 670 mg/kg body weight and 1000 mg/kg body weight doses while the 450 mg/kg body weight dose only significantly reduced mean weekly body weight gain compared to that of the normal control mice. Initially, the control and extract treated mice had similar body weights which continued up to the 14^{th} day in *B. holstii, V. lasiopus, S. asper* and *G parviflora* (at 450 mg/kg and 670 mg/kg body weight). The mice treated with *P. capense* and *G. parviflora* (at 1000 mg/kg body weight) had similar body weigh with that of the control up to the 7^{th} day. Thereafter, the control mice grew at a significantly greater rate than the extract treated mice.

Table 4.26: Effects of oral administration of aqueous plants extracts at different doses in mice daily for 28 days on body weight and the average weekly weight gain

Extract		Weekly Weight of Mice (g)					
	Treatment	0	1	2	3	4	Δ Weight/Week
B. holstii	**Control**	**23.44±1.54**	**24.74±1.51**	**26.52±1.40**	**28.46±1.19^{cd}**	**30.20±1.45^{bcd}**	**1.69±0.18^{egh}**
	450 mg/kg	23.20±0.83	24.78±0.73	25.68±0.69	26.52±0.80	27.60±0.73^{a}	**1.10±0.05^{fgh}**
	670 mg/kg	23.00±1.58	24.12±1.59	24.84±1.63	25.68±1.68^{a}	26.42±1.63^{a}	**0.86±0.05^{fe}**
	1000 mg/kg	23.40±1.14	24.20±1.22	24.92±1.07	25.86±1.22^{a}	26.66±1.16^{a}	**0.82±0.03^{fe}**
P. capense	**Control**	**23.24±1.54**	**24.82±1.39**	**26.68±1.36^{d}**	**28.30±1.24^{bcd}**	**30.02±0.98^{bcd}**	**1.65±0.39^{egh}**
	450 mg/kg	22.4±1.14	23.60±1.24	24.60±1.28	25.68±1.31^{a}	26.70±1.25^{a}	**1.08±0.07^{fh}**
	670 mg/kg	23.60±1.51	24.30±1.51	25.04±1.59	25.90±1.63^{a}	26.62±1.66^{a}	**0.76±0.07^{f}**
	1000 mg/kg	22.60±1.14	23.08±1.16	23.40±0.92^{a}	24.04±1.14^{a}	24.60±1.14^{a}	**0.50±0.03^{fe}**
V. lasiopus	**Control**	**23.60±1.14**	**25.02±1.30**	**26.68±1.30**	**28.48±1.18^{bcd}**	**30.10±1.29^{bcd}**	**1.63±0.13^{egh}**
	450 mg/kg	23.60±1.14	24.38±1.09	25.18±1.01	25.96±1.04^{a}	26.64±1.20^{a}	**0.76±0.15^{fh}**
	670 mg/kg	24.60±1.14	25.16±1.23	25.92±1.26	26.62±1.23^{a}	27.24±1.18^{a}	**0.66±0.08^{f}**
	1000 mg/kg	24.40±1.14	24.74±1.15	25.30±1.17	26.42±1.73^{a}	26.38±1.28^{a}	**0.49±0.59^{fe}**
G. parviflora	**Control**	**23.38±1.49**	**24.72±1.57**	**26.60±1.32**	**28.48±1.24^{bcd}**	**30.06±1.35^{bcd}**	**1.67±0.19^{egh}**
	450 mg/kg	22.66±1.72	23.80±1.65	24.92±1.53	25.80±1.43^{a}	26.90±1.42^{a}	**1.06±0.26^{fh}**
	670 mg/kg	23.66±1.06	24.48±1.14	25.20±1.01	25.98±1.08^{a}	26.74±1.06^{a}	**0.77±0.05^{f}**
	1000 mg/kg	23.02±1.60	23.52±1.64	24.14±1.74	24.70±1.76^{a}	25.16±1.79^{a}	**0.54±0.05^{fe}**
S. asper	**Control**	**23.80±1.14**	**24.92±1.30**	**26.70±1.67**	**28.50±1.08**	**30.06±1.30^{cd}**	**1.56±0.21^{gh}**
	450 mg/kg	24.00±1.58	25.38±1.49	26.56±1.60	27.70±1.57	28.88±1.58	**1.22±0.01^{fgh}**
	670 mg/kg	23.80±0.83	24.46±0.89	25.68±0.86	26.56±0.84	27.34±1.00^{a}	**0.89±0.05^{feh}**
	1000 mg/kg	24.40±1.14	25.10±1.12	25.74±1.08	26.36±1.12	27.08±1.14^{a}	**0.67±0.04^{feg}**

Results are expressed as Means ± Standard Deviation for five mice per group. Means accompanied by similar lower case letters in the same column are not significantly different at $\rho \leq 0.05$ by ANOVA and post ANOVA (Bonferroni-Holm) test: $^{a}\rho < 0.05$ with respect to control; $^{b}\rho < 0.05$ with respect to 450 mg/kg body weight; $^{c}\rho < 0.05$ with respect to 670 mg/kg body weight; $^{d}\rho < 0.05$ with respect to 1000 mg/kg body weight.

Results for weight change per week are expressed as Mean ± Standard Deviation for five animals in each treatment; means accompanied by similar lower case letters in the same column are significantly different by ANOVA and post ANOVA (Bonferroni-Holm) test: $^{f}\rho < 0.05$ with respect to control; $^{e}\rho < 0.05$ with respect to 450 mg/kg body weight; $^{g}\rho < 0.05$ with respect to 670 mg/kg body weight; $^{h}\rho < 0.05$ with respect to 1000 mg/kg body weight.

4.2.2 Effect of intraperitoneal administration of aqueous plants extracts on body weight and mean weekly body weight change

As shown in Table 4.27, intraperitoneal administration of aqueous extracts of *B. holstii, P. capense* and *V. lasiopus* at 1000 mg/kg body weight dose to mice daily for 28 days decreased significantly the average weekly weight gain in a dose dependent manner relative to that of the control mice. Administration of aqueous extracts of *P. capense* (at 450 and 670 mg/kg body weight) and *G. parviflora* (at 670 mg/kg body weight) to mice significantly decreased body weight as from the 21st day. Further, intraperitoneal daily administration of aqueous extracts to mice at 450 and 670 mg/kg body weight doses for *B. holstii, V. lasiopus, S. asper* and 450 mg/kg body weight for *G. parviflora* to mice significantly reduced the mean weekly body weight gain only relative to the normal control mice. However, mice treated with the aqueous extracts of *S aper* at 450 mg/kg body weight dose did not significantly alter the average weekly weight gain compared to control mice.

Table 27: Effects of intraperitoneal administration of aqueous plants extracts at different doses in mice daily for 28 days on body weight and average weekly weight gain

Extract		Weekly Weight of Mice (g)					
	Treatment	0	1	2	3	4	Δ Weight/Week
B. holstii	**Control**	**22.26±1.64**	**23.34±1.55**	**24.54±1.59**	**26.76±1.37**	**28.62±1.33^{d}**	**1.59±0.17^{egh}**
	450 mg/kg	22.80±1.31	24.24±1.13	25.32±1.40	26.16±1.30	27.14±1.21	**1.09±0.05^{fh}**
	670 mg/kg	24.00±1.41	24.94±1.28	25.80±1.18	26.64±1.11	27.44±1.09	**0.86±0.15^{f}**
	1000 mg/kg	22.60±1.14	23.44±1.16	24.10±1.17	24.72±1.22	25.28±1.14^{a}	**0.67±0.13^{fe}**
P. capense	**Control**	**22.20±1.48**	**23.42±1.18**	**24.80±1.54**	**26.72±1.30^{b}**	**28.16±1.13^{bcd}**	**1.49±0.34^{egh}**
	450 mg/kg	22.50±1.58	23.40±1.45	24.20±1.52	25.14±1.46	25.63±1.13^{a}	**0.70±0.11^{fh}**
	670 mg/kg	22.80±1.48	23.60±1.50	24.30±1.43	24.96±1.43^{a}	25.64±1.39^{a}	**0.71±0.04^{f}**
	1000 mg/kg	23.80±0.84	24.02±0.48	24.56±0.47	25.08±0.47	27.70±0.46^{a}	**0.48±0.14^{fe}**
V. lasiopus	**Control**	**22.00±1.87**	**23.20±1.58**	**24.82±1.55**	**26.62±1.35**	**28.12±1.21^{d}**	**1.53±0.37^{egh}**
	450 mg/kg	23.60±1.14	24.34±1.18	25.08±1.08	25.86±0.99	26.64±0.96	**0.76±0.15^{f}**
	670 mg/kg	24.20±0.84	24.76±0.85	25.16±1.23	26.38±0.86	27.36±0.86	**0.79±0.11^{f}**
	1000 mg/kg	23.80±0.84	24.02±0.48	24.56±0.47	25.08±0.47	27.70±0.49^{a}	**0.48±0.14^{f}**
G. parviflora	**Control**	**22.26±1.49**	**23.32±1.56**	**24.80±1.50**	**26.62±1.35**	**28.50±1.45^{d}**	**1.56±0.36^{egh}**
	450 mg/kg	23.32±0.87	24.36±0.97	25.64±0.79	26.72±0.94	27.78±0.91^{d}	**1.12±0.14^{fg}**
	670 mg/kg	23.66±0.88	24.40±0.93	25.04±0.90	25.58±0.89	26.28±0.93	**0.65±0.06^{fe}**
	1000 mg/kg	23.08±1.53	23.54±1.60	24.00±1.62	24.50±1.68	25.24±1.58^{ab}	**0.54±0.07^{fe}**
S. asper	**Control**	**22.20±1.92**	**23.02±1.72**	**25.04±1.17**	**26.54±1.36**	**27.90±1.10**	**1.43±0.40^{gh}**
	450 mg/kg	23.80±0.84	25.18±0.82	26.48±0.79	27.54±0.79	28.72±0.73	**1.23±0.04^{h}**
	670 mg/kg	24.00±1.23	24.78±1.21	25.98±1.64	26.88±1.71	27.66±1.67	**0.92±0.16^{f}**
	1000 mg/kg	24.20±1.30	24.80±1.34	25.54±1.34	26.18±1.13	26.88±1.33	**0.67±0.03^{fe}**

Results are expressed as Means ± Standard Deviation for five mice per group. Means accompanied by similar lower case letters in the same column are not significantly different at $\rho \leq 0.05$ by ANOVA and post ANOVA (Bonferroni-Holm) test: $^{a}\rho < 0.05$ with respect to control; $^{b}\rho < 0.05$ with respect to 450 mg/kg body weight; $^{c}\rho < 0.05$ with respect to 670 mg/kg body weight; $^{d}\rho < 0.05$ with respect to 1000 mg/kg body weight.

Results for weight change per week are expressed as Mean ± Standard Deviation for five animals in each treatment; means accompanied by similar lower case letters in the same column are significantly different by ANOVA and post ANOVA (Bonferroni-Holm) test: $^{f}\rho < 0.05$ with respect to control; $^{e}\rho < 0.05$ with respect to 450 mg/kg body weight; $^{g}\rho < 0.05$ with respect to 670 mg/kg body weight; $^{h}\rho < 0.05$ with respect to 1000 mg/kg body weight.

4.2.3 Effects of a oral administration of five aqueous plants extracts at different doses in mice daily for 28 days on the percent organ to body weight

Mice that received a daily oral administration of aqueous extracts of *B. holstii* and *V. lasiopus* at 450, 670 and 1000 mg/kg body weight for 28 days resulted in a significant increase in percent organ to body weight of the brain compared to that of control group (Table 4.28). Further, a daily oral administration of extracts of *P. capense* to mice at 450, 670 and 1000 mg/kg body weight for 28 days significantly increased the percent organ to body weight of the spleen compared to that of the control mice (Table 4.28). Mice treated with a daily oral administration of extracts of *G. parviflora* at 450, 670 and 1000 mg/kg body weight for 28 days showed a significantly higher percent organ to body weight of the lungs and a significantly lower organ to body weight of the kidneys compared to that of the control mice (Table 4.28). Oral administration of aqueous extracts of *S. asper* and *V. lasiopus* at 450, 670 and 1000 mg/kg body weight in mice daily for 28 days resulted in a significant reduction of percent organ to body weight and a significant elevation of percent organ to body weight of the testis, respectively compared to that of the control mice (Table 4.28).

Table 4.28: Effects of oral administration of aqueous plants extracts at different doses in mice daily for 28 days on percent organ to body weight

Extract	Treatment	Percent organ to body weight						
		Brain	Liver	Kidney	Spleen	Lungs	Testis	Heart
B. holstii	**Control**	**1.68±0.28^{bcd}**	**6.82±2.08**	**1.74±0.34**	**1.12±0.30**	**1.42±0.33**	**0.64±0.11**	**0.45±0.09**
	450 mg/kg	**2.45±0.23^{a}**	5.74±1.65	1.51±0.33	0.94±0.20	1.32±0.25	0.60±0.10	0.38±0.04
	670 mg/kg	**2.60±0.20^{a}**	5.92±0.95	1.76±0.41	1.04±0.23	1.67±0.24	0.78±0.13	0.45±0.08
	1000 mg/kg	**2.35±0.14^{a}**	5.80±0.80	1.61±0.56	0.94±0.26	1.28±0.26	0.68±0.06	0.41±0.08
P. capense	**Control**	**1.72±0.29**	**6.70±2.14**	**1.76±0.31**	**0.92±0.18^{bcd}**	**1.48±0.32**	**0.63±0.11**	**0.45±0.09**
	450 mg/kg	1.84±0.11	8.01±0.46	1.68±0.06	**1.18±0.13^{a}**	1.61±0.24	0.59±0.12	0.51±0.04
	670 mg/kg	1.64±0.10	7.37±0.85	1.59±0.22	**1.46±0.24^{a}**	1.42±0.12	0.80±0.13	0.48±0.06
	1000 mg/kg	1.99±0.02	7.01±0.69	1.78±0.14	**1.59±0.09^{a}**	1.53±0.07	0.63±0.09	0.53±0.08
V. lasiopus	**Control**	**1.46±0.17^{bcd}**	**5.83±0.13**	**1.49±0.15**	**0.96±0.18**	**1.21±0.19**	**0.55±0.06^{bcd}**	**0.39±0.06**
	450 mg/kg	**2.00±0.11^{a}**	6.87±1.15	1.44±0.41	1.23±0.29	1.38±0.28	**0.72±0.05^{a}**	0.42±0.13
	670 mg/kg	**2.05±0.19^{a}**	6.69±0.32	1.61±0.38	1.30±0.34	1.32±0.22	**0.96±0.07^{a}**	0.40±0.09
	1000 mg/kg	**2.20±0.31^{a}**	7.56±1.34	1.62±0.37	1.03±0.11	1.44±0.21	**0.81±0.18^{a}**	0.40±0.02
G. parviflora	**Control**	**1.55±0.19**	**5.89±0.71**	**1.63±0.23^{bcd}**	**1.05±0.21**	**1.27±0.18^{bcd}**	**0.60±0.08**	**0.44±0.06**
	450 mg/kg	1.73±0.13	6.28±0.80	**1.22±0.17^{a}**	0.89±0.13	**1.75±0.12^{a}**	0.58±0.08	0.43±0.03
	670 mg/kg	1.75±0.04	6.31±0.41	**1.34±0.12^{a}**	0.93±0.18	**2.44±0.39^{a}**	0.56±0.10	0.44±0.04
	1000 mg/kg	1.69±0.13	6.68±0.15	**1.35±0.13^{a}**	1.20±0.15	**1.62±0.13^{a}**	0.61±0.02	0.42±0.03
S. asper	**Control**	**1.55±0.16**	**5.90±0.68**	**1.63±0.23**	**1.05±0.14**	**1.18±0.16**	**0.62±0.08^{bcd}**	**0.43±0.03**
	450 mg/kg	1.68±0.14	6.83±0.67	1.34±0.16	1.05±0.15	1.30±0.17	**0.48±0.03^{a}**	0.42±0.02
	670 mg/kg	1.69±0.15	5.65±0.79	1.38±0.25	0.96±0.15	1.29±0.08	**0.45±0.04^{a}**	0.39±0.08
	1000 mg/kg	1.63±0.14	6.20±1.32	1.22±0.33	1.03±0.11	1.35±0.13	**0.47±0.06^{a}**	0.41±0.08

Results are expressed as Means ± Standard Deviation for five mice per group. Means accompanied by similar lower case letters in the same column are not significantly different at $\rho \leq 0.05$ by ANOVA and post ANOVA (Bonferroni-Holm) test: $^{a}\rho < 0.05$ with respect to control; $^{b}\rho < 0.05$ with respect to 450 mg/kg body weight; $^{c}\rho < 0.05$ with respect to 670 mg/kg body weight; $^{d}\rho < 0.05$ with respect to 1000 mg/kg body weight.

4.2.4 Effects of intraperitoneal administration of aqueous plant extracts at different doses to mice daily for 28 days on the percent organ to body weight

Mice intraperitoneally administered with aqueous extracts of *B. holstii* at 450, 670 and 1000 mg/kg body weight daily for 28 days resulted in a significantly higher percent organ to body weight of brain. Further, intraperitoneal administration of aqueous extracts of *B. holstii* at 670 and 1000 mg/kg body weight to mice daily for 28 days resulted in a significantly lowered organ to body weight of the heart, liver and kidney (1000 mg/kg) in comparison to the control group (Table 4.29). Intraperitoneal administration of aqueous extracts of *P. capense* at 450, 670, and 100mg/kg body weight in mice daily for 28 days resulted in a significant increase in the percent organ to body weight of the brain and spleen compared to that of the control group (Table 4.29). Further, intraperitoneally administration of aqueous extracts of *P. capense* at 450 and 670 mg/kg body weight in mice daily for 28 days resulted in a significant reduction in the percent organ to body weight of the testis compared to that of the control mice (Table 4.29).

In addition, intraperitoneal administration of aqueous extracts of *P. capense* at 1000 mg/kg body weight in mice daily for 28 days resulted in a significant decrease in the percent organ to body weight of the liver compared to that of the control group (Table 4.29). The percent organ to body weight of the brain was significantly higher in mice intraperitoneally administered with aqueous extracts of *V. lasiopus* at 450, 670 and 1000 mg/kg body weight compared to the normal control mice. In addition, there was a significant decrease in the percent organ to body weight of the liver of mice intraperitoneally administered with aqueous extracts of *V. lasiopus* at 450, 670 and

1000 mg/kg body weight daily for 28 days compared to the normal control mice (Table 4.29). Mice intraperitoneally administered with extracts of *G. parviflora* at 450, 670 and 1000 mg/kg body weight daily for 28 days resulted in a significant increase in the percent organ to body weight of the lungs compared to the control mice (Table 4.29). Further, intraperitoneal administration of aqueous extracts of *S. asper* at 450, 670 and 1000 mg/kg body weight in mice daily for 28 days resulted in a significant decrease in the percent organ to body weight of the kidney and lungs (at 1000mg/kg body weight) compared to that of the normal control mice (Table 4.29).

Table 4.29: Effects of intraperitoneal administration of aqueous extracts at different doses daily in mice for 28 days on percent organ to body weight

Extract	Treatment	Percent organ to body weight						
		Brain	Liver	Kidney	Spleen	Lungs	Testis	Heart
B. holstii	**Control**	**1.82±0.21^{bcd}**	**8.07±0.99^{cd}**	**1.73±0.37^{d}**	**1.23±0.41**	**1.46±0.14**	**0.77±0.09**	**0.48±0.07**
	450 mg/kg	**2.80±0.53^{a}**	6.41±1.34	1.67±0.45	1.02±0.23	1.59±0.17	0.65±0.08	**0.35±0.04^{a}**
	670 mg/kg	**2.43±0.19^{a}**	**5.08±0.70^{a}**	1.62±0.31	1.03±0.21	1.27±0.18	0.72±0.16	**0.36±0.04^{a}**
	1000 mg/kg	**2.36±0.23^{a}**	**5.02±0.52^{a}**	**1.30±0.14^{a}**	1.29±0.26	1.16±0.29	0.78±0.08	**0.35±0.04^{a}**
P. capense	**Control**	**1.72±0.08^{bcd}**	**8.07±0.95**	**1.81±0.39**	**0.86±0.06^{cd}**	**1.49±0.14**	**0.80±0.09^{bc}**	**0.49±0.07**
	450 mg/kg	**1.89±0.10^{a}**	7.64±0.24	1.43±0.10	1.08±0.09	1.42±0.11	**0.59±0.10^{a}**	0.50±0.04
	670 mg/kg	**1.97±0.08^{a}**	7.57±0.71	1.85±0.06	**1.22±0.29^{a}**	1.56±0.17	**0.55±0.13^{a}**	0.48±0.06
	1000 mg/kg	**1.96±0.09^{a}**	**6.46±0.45^{bcd}**	1.63±0.19	**1.92±0.08^{a}**	1.48±0.17	0.63±0.13	0.49±0.03
V. lasiopus	**Control**	**1.66±0.21^{bcd}**	**7.31±0.62**	**1.60±0.38**	**1.14±0.44**	**1.33±0.16**	**0.70±0.06**	**0.43±0.06**
	450 mg/kg	**2.03±0.08^{a}**	**5.65±0.72^{a}**	1.30±0.20	1.08±0.10	1.50±0.22	0.72±0.05	0.42±0.13
	670 mg/kg	**1.99±0.18^{a}**	**5.07±1.33^{a}**	1.52±0.24	0.94±0.23	1.54±0.36	0.72±0.06	0.42±0.07
	1000 mg/kg	**2.03±0.08^{a}**	**5.65±0.71^{a}**	1.30±0.20	1.08±0.10	1.50±0.22	.068±0.04	0.36±0.02
G. parviflora	**Control**	**1.60±0.12**	**7.48±0.59**	**1.79±0.25**	**1.19±0.23**	**1.40±0.17^{bcd}**	**0.76±0.09**	**0.46±0.06**
	450 mg/kg	1.74±0.09	7.56±0.51	1.78±0.11	0.96±0.09	**2.67±0.32^{a}**	0.75±0.22	0.44±0.05
	670 mg/kg	1.42±0.16	7.12±0.52	1.44±0.13	0.98±0.06	**1.89±0.12^{a}**	0.73±0.09	0.45±0.04
	1000 mg/kg	1.75±0.07	7.32±0.38	1.45±0.09	1.16±0.13	**1.73±0.04^{a}**	0.72±0.09	0.40±0.05
S. asper	**Control**	**1.58±0.33**	**7.31±0.33**	**1.69±0.13^{bcd}**	**1.18±0.22**	**1.43±0.10^{d}**	**0.69±0.04**	**0.43±0.04**
	450 mg/kg	1.75±0.14	6.34±1.24	**1.45±0.14^{a}**	1.07±0.18	1.39±0.05	0.62±0.12	0.45±0.04
	670 mg/kg	1.66±0.15	6.84±0.86	**1.19±0.32^{a}**	0.94±0.14	1.49±0.14	0.62±0.07	0.37±0.04
	1000 mg/kg	1.90±0.14	6.97±0.27	**1.38±0.23^{a}**	1.09±0.12	**1.26±0.13^{a}**	0.71±0.14	0.41±0.05

Results are expressed as Means ± Standard Deviation for five mice per group. Means accompanied by similar lower case letters in the same column are not significantly different at $\rho \leq 0.05$ by ANOVA and post ANOVA (Bonferroni-Holm) test: $^{a}\rho < 0.05$ with respect to control; $^{b}\rho < 0.05$ with respect to 450 mg/kg body weight; $^{c}\rho < 0.05$ with respect to 670 mg/kg body weight; $^{d}\rho < 0.05$ with respect to 1000 mg/kg body weight.

4.2.5 Effects of oral administration of aqueous plants extracts at different doses to mice daily for 28 days on hematological parameters

Oral administration of *B. holstii* aqueous plants extracts at 450, 670 and 1000 mg/kg body weight to mice daily for 28 days significantly increased levels of red blood cells, hemoglobin concentration and mean cell hemoglobin relative to the normal control mice. Further, oral administration of aqueous extracts of *P. capense* at 450, 670 and 1000 mg/kg body weight to mice daily for 28 days significantly increased platelets and the mean cell volume and decreased the mean cell hemoglobin concentration relative to that of the control mice. However, 670 and 1000 mg/kg doses of *P. capense* showed significant increase in the red blood cells. Oral administration of aqueous extracts of *V. lasiopus* at 450, 670 and 1000 mg/kg body weight to mice daily for 28 days significantly decreased packed cell volume, and the mean cell volume and increased mean cell hemoglobin concentration compared to the normal control mice. In addition, oral administration of aqueous extracts of *G. parviflora* at 450, 670 and 1000 mg/kg body weight to mice daily for 28 days resulted in a significant increase mean cell hemoglobin concentration and a decrease in packed cell volume relative to the normal control mice. Further, oral administration of aqueous extracts of *S. asper* at 450, 670 and 1000 mg/kg body weight to mice daily for 28 days resulted in a significant decrease in packed cell volume compared to the control mice (Table 4.30).

In addition, oral administration of the aqueous extracts of *B. holstii*, and *P.* capense at 450, 670 and 1000 mg/kg body weight to mice daily for 28 days resulted in a significant increase in the concentration of white blood cells, neutrophils, monocytes, lymphocytes, and eosinophils compared to the of the control mice (Table 4.31). Further, oral administration of aqueous extracts of *V. lasiopus* at

1000 mg/kg body weight to mice daily for 28 days significantly increased the concentration of white blood cells, neutrophils, monocytes, lymphocytes, and eosinophils compared to the of the control mice (Table 4.31). Oral administration of aqueous extracts of *S. asper* at 1000 mg/kg body weight to mice daily for 28 days significantly increased the concentration of white blood cells, neutrophils, monocytes, and lymphocytes compared to the of the control mice (Table 4.31). In contrast, oral administration of aqueous extracts of *G. parviflora* at 50, 670 and 1000 mg/kg body weight to mice daily for 28 days had no effect on the white blood cell count and the differential white blood cell count compared to that of the normal control mice (Table 4.31).

Table 4.30: Effects of oral administration of plants extracts at different doses daily in mice for 28 days on some end point hematological parameters in mice

Extract	Treatment	Hematological parameters and indices						
		RBC ($\times 10^6$/µL)	Hb (g/dL)	PCV (%)	MCH (pg)	MCHC (g/dL)	MCV (fL)	PLT ($\times 10^3$/µL)
B. holstii	**Control**	**7.23±1.01^{bcd}**	**10.07±2.37^{bcd}**	**50.63±3.73**	**14.70±0.83^{bcd}**	**28.52±3.04**	**52.13±4.57**	**854.76±120.33**
	450 mg/kg	**9.44±0.69^{a}**	**17.96±1.21^{a}**	53.48±5.42	**16.04±0.86^{a}**	30.80±1.27	52.02±1.62	983.40±167.72
	670 mg/kg	**9.47±0.74^{a}**	**17.14±1.12^{a}**	56.68±4.70	**16.62±1.11^{a}**	30.28±1.02	54.72±2.61	992.00±288.18
	1000 mg/kg	**9.24±0.44^{a}**	**15.52±1.21^{a}**	53.82±6.39	**17.74±0.89^{a}**	28.36±0.67	55.28±4.80	1081.40±160.59
P. capense	**Control**	**7.25±0.94**	**9.92±1.77**	**50.52±3.97**	**14.30±1.19**	**28.62±2.69^{bcd}**	**51.95±4.95^{bcd}**	**833.54±64.61^{cd}**
	450 mg/kg	7.69±0.99	12.14±1.87	48.22±2.85	15.74±0.86	**25.26±1.13^{a}**	**63.28±7.07^{a}**	1045.20±209.47
	670 mg/kg	8.54±0.54	12.00±0.98	51.42±4.04	15.26±1.53	**25.32±1.32^{a}**	**60.22±5.32^{a}**	**1210.40±252.64^{a}**
	1000 mg/kg	7.89±0.23	11.98±1.11	49.14±2.36	16.04±0.59	**25.48±1.39^{a}**	**62.98±2.46^{a}**	**1171.00±142.80^{a}**
V. lasiopus	**Control**	**6.22±0.68**	**8.64±1.74**	**49.06±2.06^{bcd}**	**14.74±0.78**	**29.50±1.14^{bcd}**	**50.30±2.22^{bcd}**	**886.00±98.85**
	450 mg/kg	6.19±1.08	9.24±2.08	**42.34±3.71^{a}**	15.34±0.40	**32.32±0.48^{a}**	**47.54±1.25^{a}**	963.42±79.32
	670 mg/kg	5.18±0.78	8.66±1.22	**39.84±1.40^{a}**	14.84±0.65	**31.08±1.14^{a}**	**47.96±1.02^{a}**	971.44±73.52
	1000 mg/kg	6.49±1.27	9.46±1.63	**40.36±3.87^{a}**	14.58±0.94	**31.46±1.07^{a}**	**46.58±2.81^{a}**	1041.62±153.56
G. parviflora	**Control**	**6.21±0.71**	**8.62±1.80**	**48.86±2.22^{bcd}**	**14.76±0.42**	**29.56±1.18^{bcd}**	**50.14±2.37**	**880.82±100.77**
	450 mg/kg	5.92±1.27	9.22±2.02	**28.50±5.70^{a}**	15.54±0.54	**32.24±0.88^{a}**	48.22±1.66	992.80±228.69
	670 mg/kg	7.00±0.86	10.96±2.17	**34.80±7.07^{a}**	16.62±0.78	**31.54±1.17^{a}**	52.74±1.86	943.80±174.47
	1000 mg/kg	6.99±0.63	9.46±2.68	**32.40±4.46^{a}**	17.12±0.67	**31.08±2.18^{a}**	54.54±1.56	1089.80±193.69
S. asper	**Control**	**6.25±0.69**	**8.36±1.70**	**49.45±1.66^{bcd}**	**14.82±1.47**	**29.48±0.72**	**50.32±1.52**	**887.20±93.21**
	450 mg/kg	6.99±1.41	10.60±2.47	**35.48±6.86^{a}**	15.10±1.03	29.68±1.35	50.86±2.34	876.40±58.84
	670 mg/kg	6.58±0.98	9.76±1.59	**38.18±5.49^{a}**	15.56±1.31	30.84±1.16	50.34±3.12	896.00±99.17
	1000 mg/kg	6.73±0.86	10.26±1.54	**33.90±5.17^{a}**	15.20±1.01	30.28±1.02	50.24±2.16	850.60±54.44

Results are expressed as Means ± Standard Deviation for five mice per group. Means accompanied by similar lower case letters in the same column are not significantly different at $\rho \leq 0.05$ by ANOVA and post ANOVA (Bonferroni-Holm) test: $^{a}\rho < 0.05$ with respect to control; $^{b}\rho < 0.05$ with respect to 450 mg/kg body weight; $^{c}\rho < 0.05$ with respect to 670 mg/kg body weight; $^{d}\rho < 0.05$ with respect to 1000 mg/kg body weight.

Table 4.31: Effects of oral administration of plant extracts at different doses daily in mice for 28 days on white blood cells and differential white blood cell count

Extract	Treatment	White blood cells and differential white blood cell count ($x10^9/L$)					
		WBC	Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils
B. holstii	**Control**	**6.80±0.43^{bcd}**	**2.16±0.20^{cd}**	**3.70±0.41^{bcd}**	**0.56±0.07^{bcd}**	**0.69±0.07^{bcd}**	**0.06±0.03**
	450 mg/kg	**10.50±2.42^{a}**	2.82±0.30	**5.42±1.07^{a}**	**0.85±0.10^{a}**	**1.07±0.04^{a}**	0.17±0.23
	670 mg/kg	**11.14±2.30^{a}**	**3.15±0.71^{a}**	**6.01±1.33^{a}**	**0.86±0.09^{a}**	**1.08±0.19^{a}**	0.06±0.06
	1000 mg/kg	**10.82±2.23^{a}**	**3.07±0.65^{a}**	**6.22±1.33^{a}**	**0.88±0.10^{a}**	**1.16±0.20^{a}**	0.06±0.07
P. capense	**Control**	**6.86±0.30^{bcd}**	**2.17±0.14^{bcd}**	**3.68±0.32^{bcd}**	**0.58±0.05^{bcd}**	**0.67±0.08^{bcd}**	**0.06±0.03**
	450 mg/kg	**12.18±3.45^{a}**	**3.37±0.92^{a}**	**6.71±1.75^{a}**	**0.85±0.25^{a}**	**1.08±0.33^{a}**	0.10±0.06
	670 mg/kg	**10.50±3.13^{a}**	**2.97±0.21^{a}**	**5.65±1.68^{a}**	**0.87±0.27^{a}**	**1.02±0.32^{a}**	0.07±0.04
	1000 mg/kg	**12.16±3.37^{a}**	**3.45±0.93^{a}**	**7.00±2.02^{a}**	**0.90±0.22^{a}**	**1.13±0.25^{a}**	0.06±0.06
V. lasiopus	**Control**	**6.88±0.63^{d}**	**2.19±0.16^{d}**	**3.75±0.35^{bcd}**	**0.59±0.05^{d}**	**0.67±0.09^{d}**	**0.06±0.03**
	450 mg/kg	8.90±0.76	2.47±0.20	**4.93±0.47^{a}**	0.63±0.10	0.77±0.13	0.05±0.04
	670 mg/kg	9.18±2.42	2.62±0.75	**4.93±0.33^{a}**	0.72±0.16	0.87±0.22	0.05±0.05
	1000 mg/kg	**13.68±1.97^{a}**	**3.79±0.49^{a}**	**7.69±1.24^{a}**	**0.86±0.19^{a}**	**0.94±0.17^{a}**	0.10±0.09
G. parviflora	**Control**	**6.78±0.44**	**2.14±0.12**	**3.66±0.29^{d}**	**0.57±0.04**	**0.67±0.08**	**0.06±0.03**
	450 mg/kg	7.02±0.65	1.96±0.25	3.88±0.33	0.52±0.66	0.61±0.05	0.04±0.04
	670 mg/kg	7.16±1.67	2.02±0.48	3.88±0.93	0.56±0.15	0.69±0.11	0.05±0.05
	1000 mg/kg	7.92±1.23	2.24±0.33	4.55±0.77^{a}	0.51±0.09	0.56±0.11	0.06±0.06
S. asper	**Control**	**6.80±0.44^{d}**	**2.15±0.16^{d}**	**3.67±0.33^{d}**	**0.56±0.06d**	**0.69±0.07**	**0.06±0.03**
	450 mg/kg	6.78±0.96	1.89±0.26	3.69±0.49	0.48±0.06	0.60±0.10	0.04±0.04
	670 mg/kg	7.22±0.85	2.03±0.18	3.88±0.43	0.57±0.12	0.71±0.14	0.05±0.04
	1000 mg/kg	**9.86±2.59^{a}**	**2.89±0.80^{a}**	**5.63±1.38^{a}**	**0.73±0.14^{a}**	0.69±0.23	0.09±0.09

Results are expressed as Means ± Standard Deviation for five mice per group. Means accompanied by similar lower case letters in the same column are not significantly different at $\rho \leq 0.05$ by ANOVA and post ANOVA (Bonferroni-Holm) test: $^{a}\rho < 0.05$ with respect to control; $^{b}\rho < 0.05$ with respect to 450 mg/kg body weight; $^{c}\rho < 0.05$ with respect to 670 mg/kg body weight; $^{d}\rho < 0.05$ with respect to 1000 mg/kg body weight.

4.2.6 Effects of intraperitoneal administration of aqueous plants extracts at different doses to mice daily for 28 days on hematological parameters

Intraperitoneal administration of aqueous extracts of *B. holstii* at 450, 670 and 1000 mg/kg body weight to mice daily for 28 days resulted in a significant increase in the levels of red blood cells, hemoglobin, mean cell hemoglobin and packed cell volume relative to that of control mice. Further, intraperitoneal administration of aqueous extracts of *P. capense* at 450, 670 and 1000 mg/kg body weight to mice daily for 28 days resulted in a significant increase in mean cell hemoglobin, mean cell volume and platelets relative to that of control mice. In addition, intraperitoneal administration of aqueous extracts of *V. lasiopus* at 450, 670 and 1000 mg/kg body weight to mice daily for 28 days resulted in a significant increase in mean cell hemoglobin concentration and a decrease in packed cell volume and mean cell volume relative to that of the normal control mice. Intraperitoneal administration of aqueous extracts of *G. parviflora* and *S. asper* at 450, 670 and 1000 mg/kg body weight to mice daily for 28 days significantly decreased packed cell volume compared to the normal control mice (Table 4.32).

In addition, intraperitoneal administration of aqueous plants extracts of *B. holstii* at 450, 670 and 1000 mg/kg body weight to mice daily for 28 days resulted in a significant increase in white blood cells, neutrophils, monocytes, lymphocytes and eosinophils compared to that of control mice (Table 4.33). Further, intraperitoneal administration of aqueous plants extracts of *P. capense* at 450, 670 and 1000 mg/kg body weight to mice daily for 28 days resulted in a significant increase in white blood cells, neutrophils, monocytes, and lymphocytes compared to that of the normal control

mice (Table 4.33). Intraperitoneal administration of aqueous plants extracts of *V. lasiopus* and *S. asper* at 1000 mg/kg body weight to mice daily for 28 days resulted in a significant increase in white blood cells, neutrophils, monocytes, lymphocytes and eosinophils compared to that of the normal control mice (Table 4.33).

Table 4.32: Effects of intraperitoneal administration of plants extracts at different doses daily in mice for 28 days on some end point hematological parameters in mice

Extract	Treatment	RBC ($\times 10^6$/µL)	Hb (g/dL)	PCV (%)	MCH (pg)	MCHC (g/dL)	MCV (fL)	PLT ($\times 10^3$/µL)
B. holstii	**Control**	**7.38±1.00^{bcd}**	**10.14±2.42^{bcd}**	**50.35±3.31^{cd}**	**14.30±0.93^{bcd}**	**28.47±2.17**	**51.54±4.41^{d}**	**882.58±46.97^{d}**
	450 mg/kg	**8.80±0.75^{a}**	**16.84±1.92^{a}**	55.60±8.43	**16.44±0.44^{a}**	30.46±1.67	54.04±1.99	1123.00±203.20
	670 mg/kg	**8.94±0.80^{a}**	**18.06±3.15^{a}**	**59.60±6.43^{a}**	**16.54±1.09^{a}**	30.30±1.28	54.58±1.83	1090.00±253.83
	1000 mg/kg	**8.94±0.96^{a}**	**16.82±1.25^{a}**	**58.16±4.20^{a}**	**16.52±0.73^{a}**	28.92±1.04	57.26±2.38^{a}	**1238.80±232.69^{a}**
P. capense	**Control**	**7.44±1.02**	**9.82±1.80**	**50.22±2.86**	**14.32±0.77^{bcd}**	**28.04±2.69**	**51.21±4.06^{bcd}**	**880.20±108.29^{bcd}**
	450 mg/kg	7.72±1.52	12.44±2.72	56.62±8.58	**16.04±0.51^{a}**	25.84±1.50	**62.28±5.47^{a}**	**1122.40±195.44^{a}**
	670 mg/kg	8.38±0.41	11.64±0.42	48.76±9.76	**16.18±0.73^{a}**	28.08±3.13	**61.20±8.01^{a}**	**1360.80±55.56^{a}**
	1000 mg/kg	8.18±1.51	11.62±2.51	45.82±8.74	**16.44±0.96^{a}**	25.22±1.03	**63.92±2.63^{a}**	**1285.40±244.88^{a}**
V. lasiopus	**Control**	**6.52±0.73**	**9.48±1.15**	**48.04±3.51^{bcd}**	**14.82±0.61**	**30.04±0.93^{bcd}**	**51.18±1.83^{bcd}**	**919.28±44.62**
	450 mg/kg	6.64±0.41	10.12±0.67	**38.56±4.01^{a}**	15.18±0.50	**33.06±0.65^{a}**	**46.10±1.85^{a}**	978.46±72.28
	670 mg/kg	5.87±0.71	7.68±1.87	**39.14±1.69^{a}**	14.54±0.33	**32.48±1.19^{a}**	**46.38±1.34^{a}**	997.66±103.20
	1000 mg/kg	6.24±0.59	9.24±0.83	**40.90±3.01^{a}**	14.78±0.73	**31.88±0.80^{a}**	**46.54±2.72^{a}**	1021.38±133.16
G. parviflora	**Control**	**7.21±1.60**	**9.64±1.13**	**47.52±3.85^{bcd}**	**14.94±0.29**	**30.08±0.95**	**50.58±2.18**	**880.74±61.11^{d}**
	450 mg/kg	6.78±1.07	9.82±1.76	**32.06±5.23^{a}**	14.48±1.19	30.60±1.44	47.66±2.69	877.60±93.74
	670 mg/kg	5.84±0.71	9.08±1.69	**28.30±5.01^{a}**	14.66±1.10	32.10±2.12	52.02±1.69	1018.20±182.51
	1000 mg/kg	6.74±2.29	9.78±1.61	**27.74±4.19^{a}**	14.52±1.70	30.40±2.19	47.56±2.32	1136.00±216.68^{a}
S. asper	**Control**	**6.38±0.84**	**9.28±1.37**	**49.45±1.66^{bcd}**	**15.02±0.57**	**29.64±1.34**	**50.98±1.62**	**919.08±46.44**
	450 mg/kg	7.08±0.80	10.88±1.33	**35.48±7.21^{a}**	15.38±1.22	30.32±1.31	50.72±3.12	892.20±66.54
	670 mg/kg	6.58±1.12	10.02±2.50	**38.64±9.12^{a}**	15.60±0.76	28.50±2.30	51.58±6.16	932.40±139.13
	1000 mg/kg	6.59±1.03	9.70±2.09	**33.88±2.65^{a}**	15.16±0.61	29.10±3.71	52.78±7.02	840.20±100.13

Results are expressed as Means ± Standard Deviation for five mice per group. Means accompanied by similar lower case letters in the same column are not significantly different at $\rho \leq 0.05$ by ANOVA and post ANOVA (Bonferroni-Holm) test: $^{a}\rho < 0.05$ with respect to control; $^{b}\rho < 0.05$ with respect to 450 mg/kg body weight; $^{c}\rho < 0.05$ with respect to 670 mg/kg body weight; $^{d}\rho < 0.05$ with respect to 1000 mg/kg body weight.

Table 4.33: Effects of intraperitoneal administration of plants extracts at different doses daily in mice for 28 days on white blood cells and differential white blood cell count.

Extract	Treatment	White blood cells and differential white blood cell count ($x10^9$/L)					
		WBC	Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils
B. holstii	**Control**	**6.88±0.50^{bcd}**	**2.23±0.28^{bcd}**	**3.64±0.45^{bcd}**	**0.56±0.07^{bcd}**	**0.64±0.75^{bcd}**	**0.06±0.03**
	450 mg/kg	**10.52±1.18^{a}**	**2.94±0.40^{a}**	**5.72±0.63^{a}**	**0.80±0.16^{a}**	**0.90±0.04^{a}**	0.09±0.05
	670 mg/kg	**11.42±2.53^{a}**	**3.17±0.73^{a}**	**6.16±1.38^{a}**	**0.87±0.21^{a}**	**1.11±0.20^{a}**	0.09±0.06
	1000 mg/kg	**10.48±1.47^{a}**	**3.01±0.42^{a}**	**5.81±0.80^{a}**	**0.78±0.18^{a}**	**0.90±0.18^{a}**	0.08±0.08
P. capense	**Control**	**6.86±0.46^{bcd}**	**2.21±0.24^{bcd}**	**3.62±0.39^{bcd}**	**0.57±0.05**	**0.64±0.06**	**0.06±0.03**
	450 mg/kg	**10.36±3.60^{a}**	**3.43±0.10^{a}**	**6.74±1.94^{a}**	0.76±0.37	0.82±0.32	0.11±0.07
	670 mg/kg	**9.72±2.18^{a}**	**3.01±0.64^{a}**	**5.23±1.12^{a}**	0.73±0.17	0.80±0.25	0.08±0.05
	1000 mg/kg	**9.42±2.07^{a}**	**2.99±0.57^{a}**	**5.28±1.23^{a}**	0.65±0.13	0.77±0.18	0.08±0.04
V. lasiopus	**Control**	**7.08±0.87^{d}**	**2.21±0.29^{d}**	**3.61±0.46^{bcd}**	**0.56±0.06^{d}**	**0.63±0.08^{cd}**	**0.07±0.03**
	450 mg/kg	8.96±2.12	2.49±0.62	**4.87±0.14^{a}**	0.69±0.16	0.78±0.16	0.08±0.05
	670 mg/kg	8.68±1.83	2.42±0.59	**4.67±0.88^{a}**	0.67±0.17	**0.85±0.18^{a}**	0.07±0.04
	1000 mg/kg	**12.74±2.49^{a}**	**3.62±0.73^{a}**	**7.12±1.31^{a}**	**0.90±0.24^{a}**	**1.04±0.23^{a}**	0.08±0.07
G. parviflora	**Control**	**6.64±0.58**	**2.20±0.31**	**3.59±0.50**	**0.56±0.07**	**0.63±0.08**	**0.06±0.03**
	450 mg/kg	7.10±1.68	1.99±0.47	3.84±0.86	0.54±0.19	0.61±0.17	0.06±0.04
	670 mg/kg	8.14±1.25	2.29±0.34	4.45±0.72	0.63±0.11	0.81±0.15	0.06±0.04
	1000 mg/kg	8.10±1.82	2.29±0.49	4.54±1.03	0.57±0.19	0.66±0.14	0.05±0.04
S. asper	**Control**	**6.70±0.57^{cd}**	**2.21±0.24^{d}**	**3.62±0.38^{cd}**	**0.57±0.05^{d}**	**0.64±0.06^{cd}**	**0.06±0.03**
	450 mg/kg	6.28±0.83	1.95±0.28	3.42±0.43	0.49±0.06	0.58±0.07	0.05±0.03
	670 mg/kg	**8.16±0.87^{a}**	2.27±0.22	**4.41±0.52^{a}**	0.61±0.05	**0.80±0.12^{a}**	0.07±0.02
	1000 mg/kg	**10.20±0.82^{a}**	**2.89±0.20^{a}**	**5.72±0.85^{a}**	**0.71±0.61^{a}**	**0.84±0.07^{a}**	0.06±0.03

Results are expressed as Means ± Standard Deviation for five mice per group. Means accompanied by similar lower case letters in the same column are not significantly different at $\rho \leq 0.05$ by ANOVA and post ANOVA (Bonferroni-Holm) test: $^{a}\rho < 0.05$ with respect to control; $^{b}\rho < 0.05$ with respect to 450 mg/kg body weight; $^{c}\rho < 0.05$ with respect to 670 mg/kg body weight; $^{d}\rho < 0.05$ with respect to 1000 mg/kg body weight.

4.2.7 Effects of oral administration of aqueous plant extracts at different doses to mice daily for 28 days on biochemical parameters

As depicted in Table 4.34, oral administration of aqueous plants extracts of *B. holstii, V. lasiopus,* and *S. asper* at 450, 670 and 1000 mg/kg body weight in mice daily for 28 days resulted in a significant elevation of the activities of Alanine transaminase (ALT) relative to values in the control mice. Oral administration of aqueous plants extracts of *P. capense, G. parviflora* and *S. asper* at 450, 670 and 1000 mg/kg body weight in mice daily for 28 days resulted in a significant reduction of the activities of Aspartate transaminase (AST) relative to values in the control mice. In addition, oral administration of aqueous plants extracts of *V. lasiopus* at 1000 mg/kg body weight in mice daily for 28 days resulted in a significant elevation of the activities of Aspartate transaminase (AST) relative to values in the control mice.

In addition, a daily oral administration of aqueous plants extracts of *B. holstii, P. capense, G. parviflora* and *S. asper* at 450, 670 and 1000 mg/kg body weight in mice daily for 28 days resulted in a significant reduction of the activities of Alkaline phosphatase (ALP) relative to values in the control mice. A daily oral administration of aqueous plants extracts of *B. holstii,* and *G. parviflora* at 450, 670 and 1000 mg/kg body weight in mice daily for 28 days resulted in a significant reduction of the activities of Lactate dehydrogenase (LDH) relative to values in the control mice. In addition, oral administration of aqueous extracts of *P. capense* at 450, 670 and 1000 mg/kg body weight in mice daily for 28 days resulted in a significant elevation of the activities of Lactate dehydrogenase (LDH) relative to values in the control mice. Oral administration of aqueous plants extracts of *V. lasiopus* and *G. parviflora* at 450, 670

and 1000 mg/kg body weight in mice daily for 28 days resulted in a significant elevation of the activities of γ-Glutamyltransferase (γ-GT) relative to values in the control mice.

Oral administration of aqueous plants extracts of *B. holstii* at 1000 mg/kg body weight and *P. capense* at 670 and 1000 mg/kg body weight in mice daily for 28 days resulted in a significant elevation of the activities of Amylase (AMYL) relative to values in the control mice. Oral administration of aqueous plants extracts of *B. holstii, P. capense, G. parviflora,* and *S. asper* at 450, 670 and 1000 mg/kg body weight in mice daily for 28 days resulted in a significant reduction of the AST/ALT ratio relative to values in the control mice.

As shown in Table 4.35, oral administration of aqueous extracts of *B. holstii, P. capense, G. parviflora* and *S. asper* at 450, 670 and 1000 mg/kg body weight in mice daily for 28 days resulted in a significant elevation of total bilirubin (T-BIL) and direct bilirubin (D-BIL) for *P. capense*, relative to values in the normal control mice. Further, oral administration of aqueous extracts of *B. holstii, P. capense, G. parviflora, V. lasiopus* and *S. asper* at 450, 670 and 1000 mg/kg body weight in mice daily for 28 days resulted in a significant reduction in blood glucose (GLU) levels, relative to values in the normal control mice.

Table 4.34: Effects of oral administration of aqueous plants extracts at different doses in mice daily for 28 days on biochemical parameters

Extract	Treatment	Biochemical parameters						
		ALT (U/L)	AST (U/L)	ALP (U/L)	GGT (U/L)	LDH (U/L)	AMYL (U/L)	AST/ALT
B. holstii	**Control**	**51.00±3.32^{bcd}**	**335.60±71.09**	**6.40±1.14^{bcd}**	**1.00±0.71**	**1690.00±153.70^{cd}**	**762.60±172.14^{d}**	**6.61±1.53^{bcd}**
	450 mg/kg	**65.60±5.13^{a}**	271.20±21.86	**3.00±1.00 ^{a}**	1.80±0.84	1469.40±181.06	902.60±179.92	**3.37±0.20^{a}**
	670 mg/kg	**67.60±3.65 ^{a}**	275.80±67.55	**3.20±1.30 ^{a}**	1.70±0.81	**1033.60±61.21 ^{a}**	962.60±168.02	**4.08±0.98^{a}**
	1000 mg/kg	**86.20±6.87 ^{a}**	250.40±55.29	**3.60±1.67 ^{a}**	2.80±1.64	**961.00±96.87 ^{a}**	**1062.60±219.16 ^{a}**	**2.90±0.59^{a}**
P. capense	**Control**	**57.60±5.50**	**357.00±71.16^{bcd}**	**7.00±1.58^{bcd}**	**1.20±0.44**	**1570.40±220.22^{bcd}**	**802.62±163.92^{cd}**	**6.32±1.84^{bcd}**
	450 mg/kg	59.40±9.88	**217.40±20.35 ^{a}**	**3.40±1.51 ^{a}**	2.40±1.14	**1935.20±71.13 ^{a}**	902.60±185.26	**3.71±0.50^{a}**
	670 mg/kg	62.60±3.64	**205.67±55.00 ^{a}**	**3.20±1.30 ^{a}**	1.80±0.83	**2142.41±89.31 ^{a}**	**1039.60±140.74 ^{a}**	**3.28±0.25^{a}**
	1000 mg/kg	47.40±9.42	**219.40±29.94 ^{a}**	**4.00±1.58 ^{a}**	2.60±1.52	**2309.80±511.41 ^{a}**	**1083.80±171.01 ^{a}**	**4.02±1.70^{a}**
V. lasiopus	**Control**	**51.20±2.77^{bcd}**	**368.60±95.11^{d}**	**7.60±1.52**	**1.20±0.44^{bcd}**	**1550.00±288.89^{d}**	**702.60±203.28^{bcd}**	**7.23±1.98**
	450 mg/kg	**78.80±10.75 ^{a}**	544.60±240.56	6.20±1.92	**2.60±0.89 ^{a}**	1986.80±360.08	**310.40±112.20 ^{a}**	6.79±2.64
	670 mg/kg	**105.80±13.82 ^{a}**	563.20±166.18	6.00±1.73	**2.80±1.30 ^{a}**	1813.20±82.85	**381.80±137.31 ^{a}**	5.47±1.89
	1000 mg/kg	**124.80±9.73 ^{a}**	**951.00±177.40 ^{a}**	6.80±1.30	**3.00±1.22 ^{a}**	**2448.00±470.46 ^{a}**	**365.40±173.15 ^{a}**	6.04±1.48
G. parviflora	**Control**	**52.00±4.84**	**355.00±63.00^{bcd}**	**6.40±1.82^{bcd}**	**1.20±0.44^{d}**	**1529.40±249.59^{cd}**	**742.60±124.35**	**6.87±1.47^{bcd}**
	450 mg/kg	52.60±3.57	**240.40±30.50 ^{a}**	**3.60±1.14 ^{a}**	2.20±1.48	**1784.40±111.35**	691.20±218.42	**4.59±0.71^{a}**
	670 mg/kg	58.00±6.20	**251.60±65.04 ^{a}**	**3.51±1.82 ^{a}**	2.10±1.61	**1858.60±136.17 ^{a}**	789.80±207.83	**4.32±0.93^{a}**
	1000 mg/kg	58.60±6.03	**240.20±80.75 ^{a}**	**3.00±1.58 ^{a}**	**2.80±0.83 ^{a}**	**1847.40±122.29 ^{a}**	782.60±140.01	**4.21±1.61^{a}**
S. asper	**Control**	**50.40±4.16^{bcd}**	**375.60±52.38^{bcd}**	**7.20±1.30^{bcd}**	**1.20±0.84**	**1750.00±254.99**	**782.61±196.73**	**7.45±0.91^{bcd}**
	450 mg/kg	**71.80±6.65 ^{a}**	**187.80±19.75 ^{a}**	**3.20±1.48 ^{a}**	0.80±0.81	1840.40±114.73	862.60±216.29	**2.61±0.16^{a}**
	670 mg/kg	**71.00±12.65 ^{a}**	**237.80±62.08 ^{a}**	**3.80±1.48 ^{a}**	1.00±0.70	1830.20±110.24	875.20±256.06	**3.40±1.06^{a}**
	1000 mg/kg	**79.80±12.11 ^{a}**	**266.20±6.41 ^{a}**	**3.04±1.64 ^{a}**	1.04±0.44	1838.64±126.04	1012.60±157.58	**3.39±0.50^{a}**

Results are expressed as Means ± Standard Deviation for five mice per group. Means accompanied by similar lower case letters in the same column are not significantly different at $\rho \leq 0.05$ by ANOVA and post ANOVA (Bonferroni-Holm) test: $^{a}\rho < 0.05$ with respect to control; $^{b}\rho < 0.05$ with respect to 450 mg/kg body weight; $^{c}\rho < 0.05$ with respect to 670 mg/kg body weight; $^{d}\rho < 0.05$ with respect to 1000 mg/kg body weight.

Table 4.35: Effects of oral administration of aqueous plants extracts at different doses in mice daily for 28 days on metabolites

Extract	Treatment	UREA (mM)	CREAT (µM)	T-BIL (µM)	D-BIL (µM)	GLU (mM)
B. holstii	**Control**	**4.90±1.93**	**44.00±7.18**	**10.04±3.22^{bcd}**	**5.76±1.63**	**5.50±0.37^{d}**
	450 mg/kg body weight	4.08±0.73	43.80±7.29	**19.36±3.49^{a}**	5.28±0.80	4.84±0.74
	670 mg/kg body weight	4.76±0.74	42.70±5.31	**24.20±2.05^{a}**	5.02±0.19	4.24±0.79
	1000 mg/kg body weight	4.96±0.70	51.80±11.71	**29.66±6.50^{a}**	6.00±0.85	**3.06±0.68^{a}**
P. capense	**Control**	**4.74±1.28**	**44.80±6.97**	**10.02±2.59^{bcd}**	**5.76±1.63^{bcd}**	**5.32±0.54^{cd}**
	450 mg/kg body weight	5.26±0.66	45.40±6.69	**26.66±2.77^{a}**	**17.66±1.39^{a}**	**4.84±0.46**
	670 mg/kg body weight	5.20±0.70	49.80±9.14	**18.98±1.08^{a}**	**13.98±2.04^{a}**	**3.64±0.82^{a}**
	1000 mg/kg body weight	5.26±0.55	46.60±6.58	**23.12±8.89^{a}**	**11.04±1.27^{a}**	**2.51±0.81^{a}**
V. lasiopus	**Control**	**5.10±1.88**	**43.80±7.29**	**9.74±0.98**	**5.54±1.20**	**5.20±0.44^{d}**
	450 mg/kg body weight	4.94±0.74	49.80±6.41	10.76±1.65	6.04±1.36	4.92±0.66
	670 mg/kg body weight	3.98±0.70	38.80±10.59	10.54±2.73	5.42±1.11	4.68±0.85
	1000 mg/kg body weight	3.70±0.94	47.60±1038	10.22±6.62	8.64±3.10	**3.58±0.87^{a}**
G. parviflora	**Control**	**5.04±1.01**	**43.80±7.29**	**10.24±3.08^{bcd}**	**5.56±1.24**	**5.32±0.54^{cd}**
	450 mg/kg body weight	5.22±1.00	44.60±7.57	**18.98±2.52^{a}**	6.98±0.75	4.68±0.77
	670 mg/kg body weight	5.26±0.68	44.51±6.42	**30.20±1.87^{a}**	5.22±0.81	**3.36±0.98^{a}**
	1000 mg/kg body weight	4.08±0.34	47.60±10.45	**20.92±3.36^{a}**	5.02±0.36	**3.48±1.00^{a}**
S. asper	**Control**	**4.94±0.58**	**48.60±8.64**	**9.06±1.15^{bcd}**	**5.38±0.89**	**5.36±0.48^{cd}**
	450 mg/kg body weight	4.92±0.43	53.62±9.61	**19.96±1.62^{a}**	6.54±2.14	4.72±0.56
	670 mg/kg body weight	5.24±0.47	55.40±9.34	**18.66±1.25^{a}**	7.08±0.50	**3.24±0.97^{a}**
	1000 mg/kg body weight	5.14±0.37	51.40±11.12	**20.26±0.65^{a}**	7.56±1.67	**3.40±0.81^{a}**

Results are expressed as Means ± Standard Deviation for five mice per group. Means accompanied by similar lower case letters in the same column are not significantly different at $\rho \leq 0.05$ by ANOVA and post ANOVA (Bonferroni-Holm) test: $^{a}\rho < 0.05$ with respect to control; $^{b}\rho < 0.05$ with respect to 450 mg/kg body weight; $^{c}\rho < 0.05$ with respect to 670 mg/kg body weight; $^{d}\rho < 0.05$ with respect to 1000 mg/kg body weight.

4.2.8 Effects of intraperitoneal administration of aqueous plant extracts intraperitoeally on biochemical parameters

As depicted in Table 4.36, intraperitoneal administration of aqueous extracts of *B. holstii,* and *V. lasiopus* at 450, 670 and 1000 mg/kg body weight and *S. asper* at 450 mg/kg body weight in mice daily for 28 days resulted in a significant elevation of alanine transaminase (ALT), relative to values in the control mice. Further, intraperitoneal administration of aqueous extracts of *P. capense* at 450, 670 and 1000 mg/kg body weight in mice daily for 28 days resulted in a significant reduction of Alanine transaminase (ALT), relative to values in the control mice. Intraperitoneal administration of aqueous extracts of *P. capense, G. parviflora,* and *S. asper* at 450, 670 and 1000 mg/kg body weight in mice daily for 28 days resulted in a significant reduction of Aspartate transaminase (ALT), relative to values in the control mice. In addition, intraperitoneal administration of aqueous extracts of *B. holstii*, *P. capense, G. parviflora* and *S. asper* at 450, 670 and 1000 mg/kg body weight in mice daily for 28 days resulted in a significant reduction of Alkaline phosphatase (ALP), relative to values in the control mice.

Intraperitoneal administration of aqueous extracts of *P. capense, V. lasiopus,* and *G. parviflora* at 450, 670 and 1000 mg/kg body weight and *B. holstii* at 1000mg/kg body weight in mice daily for 28 days resulted in a significant reduction of γ-Glutamyltransferase (γ-GT), relative to values in the control mice. Intraperitoneal administration of aqueous extracts of *B. holstii* at 450, 670 and 1000 mg/kg body weight in mice daily for 28 days resulted in a significant reduction of the activity Lactate dehydrogenase (LDH), relative to values in the normal control mice. Further,

intraperitoneal administration of aqueous extracts of *P. capense*, while *G. parviflora* at 450, 670 and 1000 mg/kg body weight in mice daily for 28 days resulted in a significant elevation of the activity of Lactate dehydrogenase (LDH) relative to values in the control mice. Intraperitoneal administration of aqueous extracts of *B. holstii* at 450, 670 and 1000 mg/kg body weight and *P. capense* at 1000mg/kg body weight in mice daily for 28 days resulted in a significant elevation in the activity of Amylase (AMYL) relative to values in the normal control mice.

In addition, intraperitoneal administration of aqueous extracts of *V. lasiopus* at 450, 670 and 1000 mg/kg body weight in mice daily for 28 days resulted in a significant reduction in the activity of Amylase (AMYL) relative to values in the normal control mice. Intraperitoneal administration of aqueous extracts of *B. holstii, G. parviflora* and *S. asper* at 450, 670 and 1000 mg/kg body weight in mice daily for 28 days resulted in a significant reduction in the ratio of AST/ALT relative to values of the control mice.

As depicted in Table 4.37, intraperitoneal administration of aqueous extracts of *B. holstii, P. capense, G. parviflora* and *S. asper* at 450, 670 and 1000 mg/kg body weight in mice daily for 28 days resulted in a significant increase in the levels of total bilirubin (T-BIL) and direct bilirubin (D-BIL) relative to values of the control mice. Further, intraperitoneal administration of aqueous extracts of *G. parviflora* at 450, 670 and 1000 mg/kg body weight in mice daily for 28 days resulted in a significant increase in the levels of urea relative to values of the control mice. Intraperitoneal administration of aqueous extracts of *B. holstii, P. capense, V. lasiopus* and *S. asper* at

450, 670 and 1000 mg/kg body weight in mice daily for 28 days resulted in a significant reduction in the levels of blood glucose relative to values of the control mice.

Table 4.36: Effects of intraperitoneal administration of aqueous plants extracts at different doses in mice daily for 28 days on biochemical parameters

Extract	Treatment	Biochemical parameters						
		ALT (U/L)	AST (U/L)	ALP (U/L)	GGT (U/L)	LDH (U/L)	AMYL (U/L)	AST/ALT
B. holstii	**Control**	**57.40±7.86^{bcd}**	**339.80±14.12**	**6.60±1.14^{bcd}**	**0.80±0.84^{d}**	**1763.80±106.65^{bcd}**	**760.40±135.56^{cd}**	**6.03±2.06^{bcd}**
	450 mg/kg	**68.00±3.39^{ad}**	279.00±10.05	**2.80±0.83^{a}**	1.80±1.48	**1259.20±124.14^{a}**	800.40±127.03	**3.32±0.14^{a}**
	670 mg/kg	**83.20±8.41^{a}**	232.80±45.40	**3.20±1.30^{a}**	2.20±1.09	**1022.00±113.73^{a}**	**1042.00±138.42^{a}**	**2.84±0.78^{a}**
	1000 mg/kg	**108.00±13.47^{ab}**	273.20±75.29	**3.00±1.20^{a}**	**2.40±1.14^{a}**	**902.00±92.83^{a}**	**1087.40±189.60^{a}**	**2.54±0.62^{a}**
P. capense	**Control**	**59.60±8.84^{d}**	**359.80±93.55^{bcd}**	**7.40±2.07^{bcd}**	**1.00±0.70^{bcd}**	**1686.80±102.84^{bcd}**	**761.80±135.58^{d}**	**6.10±1.59**
	450 mg/kg	53.20±7.79	**227.20±38.40^{a}**	**3.80±1.64^{a}**	**2.40±1.14^{a}**	**1975.40±153.64^{a}**	861.80±132.87	4.32±0.82
	670 mg/kg	45.00±11.18	**255.40±12.83^{a}**	**3.20±1.30^{a}**	**2.49±1.10^{a}**	**2059.60±124.01^{a}**	1045.80±262.10	6.00±1.69
	1000 mg/kg	**39.20±1084^{a}**	**261.60±4.77^{a}**	**3.80±1.48^{a}**	**3.20±1.30^{a}**	**2263.80±497.39^{a}**	**1141.60±168.89^{a}**	**7.08**±1.84
V. lasiopus	**Control**	**52.40±4.15^{bcd}**	**359.80±95.66^{d}**	**7.00±1.87**	**1.40±0.54^{bcd}**	**1695.80±265.63^{d}**	**700.00±192.06^{bcd}**	**6.91±1.93**
	450 mg/kg	**79.00±21.59^{a}**	544.80±203.46	6.00±1.87	**3.00±1.00^{a}**	1775.80±137.11	**265.20±11.77^{a}**	7.87±4.90
	670 mg/kg	**108.00±11.91^{a}**	586.20±206.37	5.80±1.48	**3.40±1.14^{a}**	2008.80±227.01	**426.40±108.30^{a}**	5.50±2.26
	1000 mg/kg	**114.80±21.32^{a}**	764.64±225.06^{a}	6.60±1.94	**3.20±1.64^{a}**	2383.40±446.27^{a}	**427.60±138.67^{a}**	6.72±1.82
G. parviflora	**Control**	**52.20±5.80**	**367.40±85.69^{bcd}**	**7.20±1.30^{bcd}**	**0.80±0.44^{cd}**	**1499.80±294.93^{cd}**	**700.50±192.05**	**7.20±2.37^{bd}**
	450 mg/kg	50.00±3.53	**200.00±8.80^{a}**	**4.40±1.14^{a}**	1.20±0.84	1798.60±88..85	666.38±74.34	**4.02±0.41^{a}**
	670 mg/kg	56.20±4.82	**232.80±57.74^{a}**	**4.60±1.14^{a}**	**3.20±0.83^{a}**	**1827.80±109.48^{a}**	699.80±187.89	4.86±0.93
	1000 mg/kg	60.60±2.07	**244.20±80.60^{a}**	**2.80±1.92^{a}**	**3.00±1.22^{a}**	**1875.20±153.13^{a}**	730.00±186.44	**4.06±1.41^{a}**
S. asper	**Control**	**52.00±4.58^{b}**	**363.80±88.84^{bcd}**	**7.40±1.52^{bcd}**	**1.00±0.71**	**1829.80±85.60**	**740.00±154.87**	**7.031.75^{bcd}**
	450 mg/kg	**74.20±15.49^{a}**	**191.40±8.74^{a}**	**3.60±1.14^{a}**	1.20±0.83	1823.20±122.29	780.00±124.84	**2.66±0.50^{a}**
	670 mg/kg	78.60±22.35	**207.00±8160^{a}**	**4.00±1.58^{a}**	1.40±1.14	1825.80±97.95	820.00±214.90	**4.13±2.46^{a}**
	1000 mg/kg	80.00±13.39	**268.20±17.92^{a}**	**3.20±1.30^{a}**	0.80±0.83	1827.00±112.59	962.60±165.31	**3.45±0.73^{a}**

Results are expressed as Means ± Standard Deviation for mice animals per group. Means accompanied by similar lower case letters in the same column are not significantly different at $\rho \leq 0.05$ by ANOVA and post ANOVA (Bonferroni-Holm) test: $^{a}\rho < 0.05$ with respect to control; $^{b}\rho < 0.05$ with respect to 450 mg/kg body weight; $^{c}\rho < 0.05$ with respect to 670 mg/kg body weight; $^{d}\rho < 0.05$ with respect to 1000 mg/kg body weight.

Table 4.37: Effects of intraperitoneal administration of aqueous plants extracts at different doses in mice daily for 28 days on metabolites

Extract	Treatment	Biochemical Parameters				
		UREA (mM)	CREAT (µM)	T-BIL (µM)	D-BIL (µM)	GLU (mM)
B. holstii	**Control**	**4.70±0.77**	**46.50±6.69**	**10.52±4.90^{bcd}**	**5.52±2.15**	**5.28±0.43^{d}**
	450 mg/kg body weight	4.50±0.53	54.40±10.81	**16.16±1.78^{a}**	7.60±1.53	4.98±0.49
	670 mg/kg body weight	4.76±0.46	56.40±8.64	**24.60±7.88^{a}**	6.44±1.49	3.88±0.80
	1000 mg/kg body weight	4.78±0.96	52.40±8.29	**27.92±2.81^{a}**	5.14±0.66	**3.40±0.74^{ca}**
P. capense	**Control**	**5.22±0.63**	**47.00±7.41**	**10.30±2.36^{bcd}**	**5.68±2.60^{bcd}**	**5.54±0.43^{d}**
	450 mg/kg body weight	5.28±0.59	47.40±8.56	**25.40±6.88^{a}**	**14.86±2.03^{a}**	4.72±0.19
	670 mg/kg body weight	5.20±0.90	49.60±6.38	**19.68±3.55^{a}**	**13.40±6.13^{a}**	**3.76±0.72^{a}**
	1000 mg/kg body weight	5.32±0.83	49.80±10.20	**25.30±5.70^{a}**	**11.66±1.74^{a}**	**3.51±0.82^{a}**
V. lasiopus	**Control**	**5.60±1.10**	**47.00±7.41**	**9.58±0.92**	**5.18±2.08**	**5.18±0.42^{cd}**
	450 mg/kg body weight	4.08±0.63	38.20±7.46	10.02±2.53	5.18±0.76	4.68±0.64
	670 mg/kg body weight	4.58±0.35	39.00±3.00	9.82±1.79	5.10±0.77	**3.32±0.91^{a}**
	1000 mg/kg body weight	4.02±1.20	43.20±12.67	9.34±3.98	5.52±2.54	**3.08±0.87^{a}**
G. parviflora	**Control**	**5.20±0.95^{cd}**	**47.00±7.40**	**10.72±2.63^{bcd}**	**5.58±2.49**	**5.12±0.37^{d}**
	450 mg/kg body weight	4.98±0.50	48.60±5.64	**16.04±0.91^{a}**	7.16±0.86	4.76±1.00
	670 mg/kg body weight	**4.00±0.22^{a}**	49.80±8.44	**23.26±2.67^{a}**	6.62±0.79	4.02±0.94
	1000 mg/kg body weight	**3.72±0.72^{a}**	51.00±8.94	**20.26±2.19^{a}**	5.28±0.29	3.08±0.86^{a}
S. asper	**Control**	**5.00±0.56**	**49.00±5.47**	**9.10±0.92^{bcd}**	**5.14±1.54**	**5.36±0.45^{cd}**
	450 mg/kg body weight	4.98±0.38	52.40±5.94	**17.56±3.67^{a}**	5.80±1.90	4.84±0.71
	670 mg/kg body weight	5.12±0.43	56.60±5.64	**17.98±5.64^{a}**	6.40±0.88	**3.58±0.99^{a}**
	1000 mg/kg body weight	5.22±0.55	53.80±5.02	**21.96±7.16^{a}**	7.34±1.62	**3.20±0.70^{a}**

Results are expressed as Means ± Standard Deviation for five mice per group. Means accompanied by similar lower case letters in the same column are not significantly different at $\rho < 0.05$ by ANOVA and post ANOVA (Bonferroni-Holm) test: $^{a}\rho < 0.05$ with respect to control; $^{b}\rho < 0.05$ with respect to 450 mg/kg body weight; $^{c}\rho < 0.05$ with respect to 670 mg/kg body weight; $^{d}\rho < 0.05$ with respect to 1000 mg/kg body weight.

4.3 Phytochemical composition of the five aqueous plants extracts

Table 4.38 shows that the highest and lowest concentration of flavonoids was present in aqueous extracts of *V. lasiopus* (3.81±0.05 mg/g) and *S. asper* (1.51±0.04 mg/g), respectively. The highest and lowest concentration of tannins was present in aqueous extracts of *S. asper* (3.43±0.16 mg/g) and *P. capense* (0.40±0.07 mg/g, respectively. The highest and lowest concentration of saponins was present in aqueous extracts of *B. holstii* (83.38±1.94 mg/g) and *G. parviflora* (19.78±1.31 mg/g), respectively. The highest and lowest concentration of alkaloids was present in aqueous extracts of *B. holstii* (17.27±0.94 mg/g) and *P. capense* (4.67±0.28 mg/g), respectively. The highest and lowest concentration of total phenols was present in aqueous extracts of *G. parviflora* (14.70±1.30 mg/g) and *P. capense* (4.94±0.81 mg/g), respectively. Therefore, the concentration of phytochemicals quantified varied from one medicinal plant extract to another.

Table 4.38: The concentration of the measured phytochemicals in the five aqueous plants extracts

Samples	**Phytochemical Content (mg/g)**				
	Total Phenols	**Tannins**	**Flavonoids**	**Saponins**	**Alkaloids**
B. holstii	3.61±0.50	0.80±0.12	2.15±0.04	**83.38±1.94**	**17.27±0.94**
G. parviflora	**14.70±1.30**	1.90±0.21	2.24±0.05	19.78±1.31	9.27±0.75
P. capense	4.94±0.81	0.40±0.07	1.72±0.07	35.08±1.17	4.67±0.28
S. asper	12.21±0.60	**3.43±0.16**	1.51±0.04	63.53±1.17	7.30±0.61
V. lasiopus	9.97±0.23	2.56±0.29	**3.81±0.05**	53.73±0.60	6.63±0.14

Quantities are expressed as Mean ± Standard Deviation of three determinations for each extract. The phytochemicals were expressed as mg per g of dry extract

4.4 Mineral element composition of the five aqueous plants extracts

The mineral elemental analysis of herbal extracts of *B. holstii, G. parviflora, P. capense, S. asper,* and *V. lasiopus* indicated the presence of varying levels of K, Ca, Ti, Mn, Fe, Cu, Br, Rb, Sr, Ni and Zn (Table 4.39). Aqueous extract of *B. holstii* had the highest concentrations of Ni (1.83±0.14µg/g) and Zn (143±50µg/g) while aqueous extracts of *G. parviflora* had the highest concentrations of Ca (14371±1351µg/g) and Mn (148±7µg/g). Further, aqueous extracts of *P. capense* had the highest concentrations of As (2.41±0.23µg/g), Rb (1423±73µg/g) and Pb (5.85±0.39µg/g). Aqueous extracts of *S. asper* had the highest concentrations of K (91574±4949µg/g), Ti (71.5±6.4µg/g), Cr (5.70±0.67µg/g), Fe (1218±69µg/g), Br (1317±72µg/g) and Sr (63.9±4.0µg/g). In addition, aqueous extracts of *V. lasiopus* had the highest concentrations of Cu (14.1±0.92µg/g). The aqueous extracts of *B. holstii, G. parviflora, P. capense, S. asper,* and *V. lasiopus* had K, Ti, Mn, Fe, Br, and Rb at levels above the recommended daily allowance. *P. capense* and *V. lasiopus* extracts provided Pb at levels above the recommended daily allowance for mice (Tables 4.39). While V, Se, Cd, and Hg were undetectable in all the five plants extracts, Cr (*B. holstii, G. parviflora,* and *P. capense*), Ni (*G. parviflora,* and *S. asper*), As (*B. holstii,* and *S. asper*), and Pb (*B. holstii, G. parviflora,* and *S. asper*) were undetectable in two or three of the studied aqueous plants extracts.

Table 4.39: Mineral element composition of the five aqueous plant extracts in µg/g (ppm) and concentrations administered to mice in µg

Element	Treatment	Mineral extract levels (µg/g) and amounts (µg) administered to each mouse					RDA for mice (µg/day)
		B. holstii	*G. parviflora*	*P. capense*	*S. asper*	*V. lasiopus*	
Potassium (K)		45655 ± 2372	73111 ± 2649	50112 ± 2152	**91574 ± 4949**	91263 ± 3266	$3.5x10^6$
	450 mg/kg	513.62	822.50	563.76	1030.21	1026.71	**(1250)**
	670 mg/kg	764.72	1224.61	839.38	**1533.86**	**1528.66**	
	1000 mg/kg	1141.38	**1827.78**	1252.80	**2289.35**	**2281.58**	
Calcium (Ca)		3972 ± 228	**14371 ± 1351**	1672 ± 88	7681 ± 428	2817 ± 110	$1.0x10^6$
	450 mg/kg	44.68	161.67	18.81	86.41	31.69	**(357.1)**
	670 mg/kg	66.53	240.71	28.01	128.66	47.18	
	1000 mg/kg	99.3	359.28	41.80	192.03	70.43	
Titanium (Ti)		18.6 ± 2.0	58.0 ± 4.0	24.1 ± 1.8	**71.5 ± 6.4**	44.8 ± 3.4	$3.0\ x10^2$
	450 mg/kg	**0.21**	**0.65**	**0.27**	**0.80**	**0.50**	**(0.107)**
	670 mg/kg	**0.31**	**0.97**	**0.40**	**1.20**	**0.75**	
	1000 mg/kg	**0.47**	**1.45**	**0.60**	**1.79**	**1.12**	
Vanadium (V)		< 0.20	< 0.20	< 0.20	< 0.20	< 0.20	< 0.60
	450 mg/kg	–	–	–	–	–	
	670 mg/kg	–	–	–	–	–	
	1000 mg/kg	–	–	–	–	–	
Chromium (Cr)		< 0.15	< 0.15	< 0.15	**5.70 ± 0.67**	2.50 ± 0.10	3.5x10
	450 mg/kg	–	–	–	0.06	0.03	**(12.5)**
	670 mg/kg	–	–	–	0.09	0.04	
	1000 mg/kg	–	–	–	0.14	0.06	
Manganese (Mn)		84.0 ± 3.6	**148 ± 7**	41.4 ± 3.4	94.8 ± 6.6	113 ± 5	$2.3x10^3$
	450 mg/kg	**0.95**	**1.67**	0.47	**1.07**	**1.27**	**(0.82)**
	670 mg/kg	**1.41**	**2.48**	0.69	**1.59**	**1.89**	
	1000 mg/kg	**2.10**	**3.70**	**1.03**	**2.37**	**2.83**	
Iron (Fe)		258 ± 12	1072 ± 81	334 ± 17	**1218 ± 69**	830 ± 64	$8.0x10^3$
	450 mg/kg	2.90	**12.06**	**3.76**	**13.70**	**9.33**	**(2.9)**
	670 mg/kg	**4.32**	**17.96**	**5.59**	**20.40**	**13.90**	
	1000 mg/kg	**6.45**	**26.80**	**8.35**	**30.45**	**20.75**	
Nickel (Ni)		**1.83 ± 0.14**	< 0.050	1.25 ± 0.13	< 0.050	1.44 ± 0.14	$< 1.0x10^3$
	450 mg/kg	0.02	–	–	–	0.02	**< (0.36)**
	670 mg/kg	0.03	–	0.02	–	0.02	
	1000 mg/kg	0.05	–	0.03	–	0.04	

Copper (Cu)	11.2 ± 0.70	4.86 ± 0.45	8.33 ± 0.50	6.05 ± 0.65	**14.1 ± 0.92**	1.5x10^3
450 mg/kg	0.13	0.06	0.09	0.07	0.16	**(0.54)**
670 mg/kg	0.19	0.08	0.14	0.10	0.24	
1000 mg/kg	0.28	0.12	0.21	0.15	0.35	
Zinc (Zn)	**143 ± 50**	56.7 ± 2.60	114 ± 60	51.9 ± 3.40	55.4 ± 2.50	1.1x10^4
450 mg/kg	1.61	0.64	1.28	0.58	0.62	**(3.9)**
670 mg/kg	2.39	0.95	1.91	0.87	0.93	
1000 mg/kg	3.58	1.41	2.85	1.29	1.39	
Arsenic (As)	< 0.02	0.910 ± 0.06	**2.41 ± 0.23**	< 0.02	1.15 ± 0.13	1x10^3
450 mg/kg	_	0.01	0.03	_	0.01	**(0.357)**
670 mg/kg	_	0.01	0.04	_	0.02	
1000 mg/kg	_	0.02	0.06	_	0.03	
Selenium (Se)	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	7.5x10
450 mg/kg	_	_	_	_	_	**(0.00107)**
670 mg/kg	_	_	_	_	_	
1000 mg/kg	_	_	_	_	_	
Bromine (Br)	51.0 ± 1.90	556 ± 21	16.6 ± 1.20	**1317 ± 72**	174 ± 13	1x10^3
450 mg/kg	**0.57**	**6.26**	0.19	**14.82**	**1.96**	**(0.357)**
670 mg/kg	**0.85**	**9.31**	0.28	**22.06**	**2.91**	
1000 mg/kg	**1.28**	**13.90**	**0.42**	**32.93**	**4.35**	
Rubidium (Rb)	134 ± 9	11.3 ± 0.69	**1423 ± 73**	31.8 ± 2.1	43.0 ± 1.9	1x10^3
450 mg/kg	**1.51**	0.12	**16.01**	0.36	**0.48**	**(0.357)**
670 mg/kg	**2.24**	0.19	**23.83**	**0.53**	**0.72**	
1000 mg/kg	**3.35**	0.28	**35.58**	**0.79**	**1.08**	
Strontium (Sr)	31.1 ± 2.5	57.1 ± 2.5	17.7 ± 0.7	**63.9 ± 4.0**	27.9 ± 1.3	
450 mg/kg	0.35	0.64	0.19	0.72	0.31	
670 mg/kg	0.52	0.96	0.29	1.07	0.47	
1000 mg/kg	0.78	1.43	0.44	1.59	0.69	
Cadmium (Cd)	< 0.10	< 0.10	< 0.10	< 0.10	< 0.10	1x10^3
450 mg/kg	_	_	_	_	_	**(0.357)**
670 mg/kg	_	_	_	_	_	
1000 mg/kg	_	_	_	_	_	
Mercury (Hg)	< 0.03	< 0.03	< 0.03	< 0.03	< 0.03	
450 mg/kg	_	_	_	_	_	
670 mg/kg	_	_	_	_	_	

Lead (Pb)	1000 mg/kg	_	_	_	_	_		
		< 0.03	< 0.03	**5.85 ± 0.39**	< 0.03	0.69 ± 0.03	$1x10^3$	
	450 mg/kg	_	_	0.07	_	0.01	**(0.357)**	
	670 mg/kg	_	_	0.10	_	0.01		
	1000 mg/kg	_	_	0.15	_	0.02		

Results are expressed as Mean ± standard deviation; < means below the limit of detection of TXRF/AAS. Results on the concentration of each mineral are expressed as µg/g of the extract for each of the five studied plants. The first row of each mineral indicates mineral levels in the plant extract in µg/g while the second, third and fourth indicates the daily mineral administered (µg/day). This is compared with the recommend daily allowance shown in the last column.

CHAPTER FIVE

DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 DISCUSSION

The present work was undertaken inorder to investigate the antidiabetic activity and safety of the aqueous extracts of *B.holstii, P. capense, V. lasiopus, G. parviflora and S.asper* in alloxan-induced diabetic mice. Alloxan-induced diabetic mice had a 2.6- to 4.6-fold increase in blood glucose (13 to 23mM) relative to the normal control mice (5mM). This increase in blood glucose level after alloxan treatment occurs because alloxan destroys and reduces the pancreatic β-cells via formation of reactive oxygen species like nitric oxide (Skudelski, 2001). This destruction affects insulin synthesis and release making it unavailable or insufficient and thus inducing type I diabetic state (Arika *et al.,* 2016).

Oral and intraperitoneal administration of the aqueous extracts of *B. holstii, P. capense, V. lasiopus, G. parviflora* and *S. asper* at 25, 48.4, 93.5, 180.9 and 350 mg/kg body weight in diabetic mice demonstrated blood glucose lowering effect in a dose independent manner indicating that these extracts contained hypoglycemic constituents. This blood glucose lowering effect of these five plants extracts is similar to other plants extracts studied and reported by other researchers previously (Shaw *et al*., 2010). The hypoglycemic effect of the five aqueous plants extracts of *B. holstii, P. capense, V. lasiopus, G. parviflora* and *S. asper* when administered either intraperitoneally or orally in five doses in diabetic mice in a dose independent manner may reflect uptake of the active constituents through saturable active transport, or it

may also reflect maximum hypoglycemic activity at the lowest dose used (25 mg/kg body weight). The return to hyperglycemic state in the the twenty-fourth hour after treatment with the aqueous regardless of the route of administration helps to define the frequency of dosing and is due to the reduction of the hypoglycemic constituents in circulation. This may be a result of first-pass hepatic metabolism and renal clearance or degradation based on the short half-life of the hypoglycemic constituents (Mukundi *et al.,* 2015).

The blood glucose lowering potential of these plants was in tandem with reported literature of other plants already studied. Ethanolic and water extracts of *Caesalpinia bonducella* exhibited hypoglycemic activity through enhancement of insulin secretion in isolated inslets, when administered in chronic type II diabetics (Piero *et al*., 2012). Aqueous extract of *Tribuluks terrestris* significantly lowered the blood glucose levels in both normal and alloxan-induced diabetic mice, through enhanced secretion of serum insulin (Abdirahaman *et al.,* 2015). Propanone extract of *Elephantopus scaber* administered in streptozotocin-induced diabetic rats, demonstrated a significant lowering of blood glucose through improvement of insulin sensitivity, revitilization of glucose dependent insulin secretion as well as enhanced regeneration of pancreatic islets (Abdirahaman *et al*., 2015).

The higher rate constants and shorter half-lifes of the five aqueous plants extracts when administered intraperitoneally at 25, 48.4, 93.5, 180.9 and 350 mg/kg body weight in diabetic mice relative to orally administered extracts could be because the

intraperitoneal route provides the hypoglycemic constituents immediately into the systemic circulation in high concentrations. In the oral route, the active hypoglycemic constituents' concentration reduced before reaching the systemic circulation through the first-pass liver metabolism or had limited gastrointestinal absorption via the gut mucosal epithelial cells or the hypoglycemic constituents were transported slowly across the intestinal wall. Orally administered active constituents are absorbed by the gut mucosal epithelial cells and enter the hepatic portal system, which transports them through the portal vein into the liver where substantial catabolism occurs before release of reduced levels of uncatabolized active constituents into systemic circulation (Michael, 2010).

Of all the five studied aqueous plants extracts, those of *P. capense* and *G. parviflora* at at 25, 48.4, 93.5, 180.9 and 350 mg/kg body weight demonstrated the highest hypoglycemic activity in diabetic mice. This implies that either these two extracts contained the highest concentration of the hypoglycemic constituents or they contained the most active hypoglycemic constituents or contained active hypoglycemic constituents with longer half-lifes. These high levels of the hypoglycemic constituents in these two plants could be due to differences in the soil composition and the environmental stress at the sites where these plants were growing and harvested. Bioactive constituents of plants are secondary products produced to help them survive the stress induced to them by the environment. They are products of inducible genes (Nyamai *et al.,* 2016).

The blood glucose lowering effect of these five plants extracts may be attributed to the presence of total phenols, flavonoids, alkaloids, tannins and saponins that have been associated with hypoglycemic activity (Elliot *et al*., 2000). The hypoglycemic effects of aqueous extracts of *B. holstii, P. capense, V. lasiopus, G. parviflora* and *S. asper* could be due to the presence of total phenols, alkaloids, flavonoids, tannins, and saponnins. As reported by Ghule *et al*. (2006), flavonoids like myricetin, a polyhydroxylated flavonol has insulinomimetic properties and stimulate lipogenesis and glucose transport in the adipocytes hence lowering blood sugar (Elliot *et al*. 2000). Flavonoids stimulate transport of glucose in the adipocytes and lipogenesis, hence decreasing blood glucose. Flavonoid glycosides of *Psidium guajava* such as strictinin, isostrictinin and pedunculagin manage diabetes by improving the sensitivity of insulin (Abdirahman *et al*., 2015). Flavonoid fractions from *Pterocarpus marsupium* cause pancreatic beta cell regulation (Arika *et al*., 2015). Epicatechin and catechin flavonoids from *Pterocarpus marsupium* have been reported to demonstrate antidiabetic properties (Subramanian, 1981).

Kimura and Suzuki (1985) demonstrated that saponins lowered blood glucose in diabetic and normal mice. Abdirahman *et al*. (2015) reported that total saponins from the seeds of *Entada phaseoloides* reduced fasting blood glucose and alleviated hyperglycemia in type II diabetic rats. Sui *et al*. (1994) reported that a saponin isolated from the leaves of *Acanthopanax senticosus* injected into mice (100, 200 mg/kg body weight, intraperitoneally) decreased experimental hyperglycemia induced by injection of adrenaline, glucose and alloxan, without affecting the levels of blood sugar in

untreated mice. Oral administration of *Citrullus colocynthis* on normalglycemic rabbits at a dose of 50 mg/kg body weight demonstrated that a saponin-component reduced glycemia after 1, 2, 3 and 6 hour. Graded doses of saponin extract (10, 15 and 20 mg/kg body weight) caused a marked hypoglycemic effect in alloxan-induced diabetic rabbits (Abdel-Hassan *et al*., 2003).

The alkaloid, 1-ephedrine promotes the regeneration of pancreas islets following destruction of the beta cells, hence restores the secretion of insulin, and thus corrects hyperglycemia (Elliot *et al*., 2000). The tannin epigllo-catechin-3-gallate exhibits antidiabetic activity as demonstrated by Broadhurst *et al*. (2000).

The hypoglycemic effect of these five plants extracts could also be due to the observed presence of trace elements such as iron, chromium, manganese, zinc, copper, potassium and calcium (Mooradian *et al*., 1994). Iron, present in aqueous extracts of *B. holstii*, *P. capense*, *V. lasiopus*, *G. parviflora* and *S. asper* influences glucose metabolism and reciprocally, iron influences insulin action. Iron interferes with insulin inhibition of glucose production by the liver (Niederau *et al*., 1984).

Chromium, present in aqueous extracts of *V. lasiopus*, and *S. asper*, functions as a cofactor in insulin-regulating activities. It facilitates insulin binding and subsequent uptake of glucose into the cell and therefore decreases fasting glucose levels, improves glucose tolerance, lowers insulin levels and decreases total cholesterol in type II diabetic subjects (Baker, 1976; Mooradian *et al*., 1994). Manganese, present in

aqueous extracts of *B. holstii*, *P. capense*, *V. lasiopus*, *G. parviflora* and *S. asper*, is an activator and constituent of several enzymes like kinases and enzymes of oxidative phosphorylation (Friedman *et al*., 1987). Zinc, present in aqueous extracts of *B. holstii*, *P. capense*, *V. lasiopus*, *G. parviflora* and *S. asper*, plays a key role in the regulation of insulin production by pancreatic tissues and glucose utilization by muscles and fat cells (Song *et al*., 1998). Zinc also influences glyceraldehyde-3-phosphate dehydrogenase, the enzyme involved in glycolysis (Jose' Manuel *et al*., 2002).

Copper, present in aqueous extracts of *B. holstii, P. capense, V. lasiopus, G. parviflora* and *S. asper,* promotes lipogenesis because it has insulin like-like activity (Rajendra *et al.,* 2007). Deficiency of copper results in lack of glucose tolerance. It is associated with atherosclerosis and hypercholesterolemia (Abdirahman *et al.,* 2015). Potassium, present in aqueous extracts of *B. holstii, P. capense, V. lasiopus, G. parviflora* and *S. asper,* improves insulin sensitivity, and secretion. Depletion of potassium can result in glucose intolerance (Rajendra *et al.,* 2007).

Calcium, present in aqueous extracts of *B. holstii, P. capense, V. lasiopus, G. parviflora* and *S. asper,* improves sensitivity of insulin in some type II diabetic population (Rajendra *et al.,* 2007). The increased concentration of cytosolic calcium mediates the effect of glucose by stimulating release of insulin from islet of Langerhans of rat (Abdirahman *et al.,* 2015). Nickel deprivation decreases reproductive performance, plasma glucose and growth and alters the distribution of

other elements in the body, including calcium, and zinc as reported in rats and human studies (Piero *et al.,* 2012). Nickel was present in aqueous extracts of *B. holstii, P. capense,* and *V. lasiopus*.

Toxicity studies are essential in assessment of safety of extracts or drugs used in clinical medicine. Chronic toxicity data predicts the hazard of long-term, low-dose exposure to a particular compound; this may cause significant changes in the structure, function, metabolic transformation and concentration of biomolecules, enzymes and even metabolic pathways. These alterations may be rapid or slow and may lead to different biochemical mechanism producing similar pathological, clinical and laboratory findings (Kaku *et al.* 1995). Acute toxicity evaluation is also important in assessing immediate effects of the administered substances. Therefore, because the toxicity of a drug to the bystander host cells could render it unsuitable for therapeutic purposes, the toxicity of high doses of the five aqueous plants extracts exhibiting hypoglycemic activity was studied in normal mice.

Oral and intraperitoneal administration of aqueous extracts of *B. holstii, P. capense, V. lasiopus, G. parviflora* and *S. asper* at 450, 670 and 1000mg/kg body weight to normal mice daily for 28 days resulted in a significant sequential dose dependent reduction of the average weekly body weight indicating the presence of constituents in the extracts that retard growth. This decrease in average weekly body weight gain could be due to iron levels beyond the recommended daily allowances present in these aqueous plants extracts, which are associated with a low growth rate. High iron levels

cause hemochromatosis, characterized by weakness, weight loss among others. The lack of remarkable increase in average weekly body weight in various experimental groups relative to the control group indicates the inability of these plants extracts to control muscle wasting. Antinutrients, present in these aqueous extracts, may inhibit nutrient and mineral absorption and therefore induce the reduced growth rate in the experimental groups relative to the control group. Such antinutrients include phytates, which inhibit absorption of non-hem iron. However, antinutrients levels were not determined in these aqueous extracts. Phytase, the enzyme that degrades phytates in plant material is destroyed at the 60^{o}C extraction temperature of the aqueous plants extracts.

Concerning changes in body weight, a loss of body weight of more than 10% such as that induced by *B. holstii* (Oral [670 and 1000 mg/kg body weight], IP [1000 mg/kg body weight]), *P. capense* [450, 670 and 1000mg/kg body weight], *V. lasiopus* [450, 670 and 1000 mg/kg body weight], and *G. parviflora* (Oral [450, 670 and 1000 mg/kg body weight], IP [1000 mg/kg body weight]) is associated with protein-energy malnutrition. This is associated with impaired physiological function such as impaired cell mediated and humoral immunity. Such constituents may include components such as alkaloids, flavonoids and tannins present in these aqueous extracts in addition to other undetermined constituents.

Alkaloids such as ρ-octopamine and synephrines may reduce body weight by exerting adrenergic agonist activity. Synephrines increase energy expenditure (EE) (resting

energy expenditure [70%], thermic effect of feeding [10%], and energy expenditure of physical activity [20%]) and decrease food intake in addition to decreasing gastric motility (slows gastric empting and intestinal transit) (Tucci, 2010, Astrup 2000) and indirectly producing increased feeling of satiety and a decreased appetite. Nicotine an alkaloid induces weight loss by exerting its effects through the central nervous system and metabolic actions by reducing appetite and altering feeding patterns. It increases metabolic rate, increases energy expenditure (EE), and hence decreases metabolic efficiency (Tucci, 2010). In the CNS, nicotine modulates the central nervous system pathways that regulate several aspects of food intake (Tucci, 2010).

The flavonoid, chlorogenic acid reduces body weight by inducing reduction in body fat by reducing the absorption of glucose (energy source) leading to an increase in the consumption of fat reserves. A major consequence of blocking digestion of carbohydrates in the proximal gut is colonic fermentation, which leads to increased microbial production of gas in the bowl; gas production limits glucose utilization (Thom 2007; Tucci 2010). Catechins (flavonoids) such as epigallocatechin gallate, epigallocatechin, epicatechin gallate, and epicatechin are associated with increase in sympathetic nervous system activity, thermogenesis, and fat oxidation and hence accounting for the reduced body weight (Dulloo et al., 1999; Nagao et al., 2000). Catechins also induce reduction of body fat by inhibiting small intestine micelle formation and inhibiting α-glucosidase activity leading to decreased carbohydrate absorption (Tucci 2010; Muramatsu et al. 1986). Caffeine, a flavonoid induces weight loss by decreasing energy intake (EI) (Jessen *et al.*, 2005) by acting through the

central and peripheral nervous system mechanisms and by promoting thermogenesis and lipolysis (Acheson *et al.,* 1980; Tucci 2010). The central nervous system effects of caffeine are due to its effects on the widely distributed adenosine α1, α2A, and α2B receptors (Fredholm *et al.,* 1999, Quarta *et al.,* 2004).

For the oral route, tannins, which are present in high amounts in these five aqueous extracts may reduce feed intake by decreasing palatability and by reducing feed digestion. Palatability reduces because tannins are astringent. Astringency is the sensation caused by the formation of complexes between tannins and salivary glycoproteins. Low palatability depresses feed intake. Digestibility reduction negatively influences intake because of the filling effect associated with undigested feedstuff. Tannins consist of hydrolysable and condensed tannins. Hydrolysable tannins are convertable by microbial metabolism and gastric digestion into absorbable low molecular weight metabolites such as tannic acid, which are toxic. The major lesions associated with hydrolysable tannins poisoning are hemorrhagic gastroenteritis, which decreases absorption of nutrients.

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

Overdose of minerals such as K, Ti, Mn, Fe, Rb and Br may also cause toxicity; these minerals were beyond the recommended daily allowance in the five aqueous extracts of *B. holstii, P. capense, V. lasiopus, G. parviflora* and *S. asper*. The excess potassium present in aqueous extracts of *G. parviflora, V. lasiopus* and *S. asper* at 670, and 1000 mg/kg body weight in normal mice leads to hyperkalemia. Hyperkalemia can also result from either a shift of potassium from cells to the extracellular fluid (ECF) or excessive potassium retention, which may be caused by a major trauma and infection, metabolic acidosis, Addison's disease (aldosterone insufficiency) and chronic renal failure. Overuse of potassium supplements can also result in potassium excess. The most important clinical consequence of potassium excess is cardiac arrest (Strain and Cashman, 2009); however, there was no sinlge death observed in experimental mice used in this safety study. That no death occurred may be due to the close metabolic interactions among the major electrolytes, potassium and sodium, which are important in determining the risk of coronary heart disease and stroke. Another potentially important interaction is calcium. Potassium has positive effects on calcium balance by regulating the acid-base balance and ameliorating effects of sodium on calcium depletion (Strain and Cashman, 2009).

Titanium is a non-poisonous metal and humans can tolerate large quantities of titanium. However, rats exposed to titanium dioxide through inhalation develop localized areas of dark-coloured dust deposits in the lungs. Slight anatomical changes of human lungs may occur from excessive titanium exposure (Meltzer, 1997).

The excess manganese present in aqueous extracts of *B. holstii, V. lasiopus, G. parviflora* and *S. asper* at 450, 670, and 1000 mg/kg body weight and *P. capense* at 1000 mg/kg body weight in normal mice causes manganic madness manifested by psychosis, hallucinations and extrapyramidal damage with features of Parkinsonism. Excess manganese causes iron to accumulate in the extracellular fluid. Excess manganese inhibits dietary iron absorption because of competition for similar binding and absorption sites between nonheme iron and manganese (Strain and Cashman, 2009). This competition of manganese and iron may explain the absence of psychosis, hallucinations and extrapyramidal damage in the experimental mice in this safety study.

Excess iron causes cellular and tissue injury; this may account for the enlargement of lungs [*G. parviflora* (Oral, IP), testis [*V. lasiopus* (Oral), brain [*B. holstii* (Oral, IP); *V. lasiopus* (Oral, IP), and spleen [*P. capense* (Oral, IP) of mice treated with aqueous extracts at 450, 670 and 1000 mg/kg body weight daily for 28 days. Excess iron uptake inhibits manganese uptake since the uptake of one antagonizes the uptake of the other. Excess iron could lead to increased risks for bacterial infection, neoplasia, arthropathy, cardiomyopathy, and endocrine dysfunctions. Excess iron uptake implies excess copper uptake; the two enter into the cells together. The uptake of one accompanies the uptake of the other (Strain and Cashman, 2009).

Rubidium is non-harmful to humans. Rubidium ion excretion occurs rapidly in sweat and urine. In humans, rubidium chloride is used to study the transport of potassium

ions. This is because rubidium ions are absent in the human body; however, when present they are treated as potassium. Partial substitution of potassium with rubidium is possible; however, if more than 50% of potassium is replaced with rubidium in rats, they die (Meltzer, 1997). Rubidium treated rats significantly secrete increased levels of of N-acetyl-beta-D-glucosaminidase (NAG) in their saliva. (NAG) is a lysosomal enzyme usually released following damage to submandibular gland (Baharnouri, 1998). Some compounds of bromine ($KBrO_3$) have been associated with nephrotoxicity and carcinogenity in rodents (Geter, 2006). Other studies have demonstrated altered lipid metabolism and lipid perixidation following exposure to potassium bromate (Chipman *et al.*, 1998).

The increase in red blood cells count, hemoglobin concentration, packed cell volume, and/or mean cell hemoglobin caused by a daily oral and intraperitoneal administration of aqueous extracts of *B. holstii* at 450, 670 and 1000mg/kg body weight in normal mice for 28 days could be due to dehydration or due to an abnormal increase in white blood cell production by the bone marrow caused by some of the extract constituents. This leads to tissue hypoxia. The significant increase in packed cell volume may be attributed to excessive production of reticulocytes (polycythemia). This may imply that the plant extract aided in the formation or release of erythropoietin, which activates the stem cells to produce red blood cells by the bone marrow (Messier and Ohls, 2014).

The increase in mean cell hemoglobin and mean cell volume caused by a daily intraperitoneal administration of aqueous extracts of *P. capense* at 450, 670 and 1000mg/kg body weight in normal mice for 28 days could be due to dehydration or due to an abnormal increase in white blood cell production by the bone marrow caused by some of the aqueous extracts constituents. This induces hyperchromic macrocytic anemia which leads to tissue hypoxia. Hyperchromic macrocytic anemia could be due to folate or vitamin B_{12} deficiency or red blood cell hemolysis induced by aqueous extracts constituents. Folate and/or vitamin B_{12} deficiency could be confirmed by assessing the serum levels of these vitamins which was not carried out in this study. The significant increase in mean cell volume could be associated with enlarged red blood cells which hamper transport of oxygen in tissues and leads to tissue hypoxia.

The decrease in mean cell hemoglobin and increase in mean cell volume caused by a daily oral administration of aqueous extracts of *P. capense* at 450, 670 and 1000mg/kg body weight in normal mice for 28 days indicates that this extract contains constituents that induce hypochromic macrocytic anemia which leads to tissue hypoxia.

The decrease in packed cell volume, and/or mean cell hemoglobin caused by a daily oral and intraperitoneal administration of aqueous extracts of *G. parviflora* and *S. asper* at 450, 670 and 1000mg/kg body weight in normal mice for 28 days indicates that these extracts contain constituents that induce normochromic normocytic anemia and microchromic normocytic anemia, respectively, both of which lead to tissue

hypoxia. This could be due to iron deficiency which could have been confirmed by assessing the serum ferritin levels. This was not carried out in this study. However, the sequential decrease in average weekly weight gain with the increase in extract doses suggests reduced feed and nutrient intake which could lead to iron deficiency.

The decrease in packed cell volume, mean cell volume and the increase in mean cell haemoglobin concentration caused by a daily oral and intraperitoneal administration of aqueous extracts of *V. lasiopus* at 450, 670 and 1000mg/kg body weight in normal mice for 28 days indicates that these extracts contain constituents that induce hyperchromic macrocytic anemia which leads to tissue hypoxia. These abnormal levels of blood parameters leading to different blood conditions are due to the presence of toxic constituents in the aqueous plants extracts such as alkaloids, flavonoids, tannins and saponins previously reported to reduce erythron parameters (Barger, 2003).

Tissue hypoxia causes most tissues such as brain (*B. holstii* [oral, IP], *P. capense* [oral], *V. lasiopus* [oral, IP]), spleen (*P. capense* [oral, IP]), testis (*V. lasiopus* [oral]), and lungs (*G. parviflora* [oral, IP]) to initially enlarge and as the swollen cells continue rupturing, the organs such as liver (*B. holstii* [oral, IP], *V. lasiopus* [oral]), kidney (*B. holstii* [IP], *G. parviflora* [oral], *S. asper* [IP]), heart (*B. holstii* [IP]), lungs (*S. asper* [IP]), and testis (*P. capense* [IP], *S. asper* [oral]) reduce in size (organ atrophy) (Voet and Voet 2004). During tissue hypoxia, cells which rely only on glycolysis for ATP production rapidly deplete the store of phosphocreatine (a source

of rapid ATP production) and glycogen. As the rate of ATP production decreases below the level required by membrane ion pumps for the maintenance of proper intracellular ionic concentrations, the osmotic balance of the cell is disrupted so that the cell and its membrane enveloped organelles swell. The overstretched membrane becomes permeable thereby leaking their enclosed contents. The decreased intracellular pH that accompanies anaerobic glycolysis because of lactic acid production permits the released lysosomal enzymes which are only active at acidic pH to degrade the cell contents. Among the degraded components are the initially elevated serum enzymes which are later reduced to values below the control values as the hypoxic state progresses. The reduced metabolic activity results in irreversible cell damage (Voet and Voet 2004).

Injury of organs resulting from tissue hypoxia may also partly account for the increased serum levels of alanine transaminase (liver; *B. holstii* [oral, IP], *V. lasiopus* [oral, IP], *S. asper* [oral, IP]), alkaline phosphatase (*B. holstii* [oral]), amylase (pancreas; *B. holstii* [oral, IP], *P. capense* [oral, IP]), γ-glutamyltransferase (liver; *P. capense* [IP], *V. lasiopus* [oral, IP]), total bilirubin (liver; *B. holstii* [oral, IP], *P. capense* [oral, IP], *G. parviflora* [oral, IP], *S. asper* [oral, IP]), direct bilirubin (liver; *B. holstii* [oral, IP], *P. capense* [oral, IP], *G. parviflora* [oral, IP], *S. asper* [oral, IP]) orally and intraperitoneally administered with aqueous extracts of *B. holstii*, *P. capense*, *V. lasiopus*, *G. parviflora* and *S. asper* at 450, 670 and 1000mgkg body weight in normal mice daily for 28 days (Boyd, 1983; Clampitt and Hart, 1978; Doxey, 1984; Kaneko, 1989; Moss and Handerson, 1999).

Further, injury of organs resulting from tissue hypoxia may also partly account for the decreased levels of lactate dehydrogenase (liver, kidney and heart; *B. holstii* [oral, IP], *P. capense* [oral]), alkaline phosphatase (*B. holstii* [IP], *P. capense* [oral, IP], *G. parviflora* [oral, IP], *S. asper* [oral, IP]), aspartate transaminase (liver, kidneys, heart and pancreas; *P. capense* [oral, IP], *G. parviflora* [oral, IP], *S. asper* [oral, IP]), amylase (pancreas; *V. lasiopus* [oral, IP]), glucose (*B. holstii* [IP], *P. capense* [oral, IP], *V. lasiopus* [oral, IP], *G. parviflora* [oral, IP] and *S. asper* [oral, IP]) and aspartate transaminase/alanine transaminase ratio (*B. holstii* [oral, IP], *P. capense* [oral, IP], *V. lasiopus* [oral, IP], *G. parviflora* [oral, IP] and *S. asper* [oral, IP]) orally and intraperitoneally administered with aqueous extracts of *B. holstii, P. capense, V. lasiopus, G. parviflora* and *S. asper* at 450, 670 and 1000mgkg body weight in normal mice daily for 28 days (Boyd, 1983; Clampitt and Hart, 1978; Doxey, 1984; Kaneko, 1989; Moss and Handerson, 1999).

The observed organ damage could also be due to the phytochemicals present in these aqueous extracts. Saponins hemolyse red blood cells and cause cell death to many tissues (Al-Sultan *et al.*, 2000, Diwan, 2000). In the kidneys, saponins lead to haemorrhage in the glomeruli and focal destruction of the renal tubules. Toxic levels of saponins cause cardiac failure, acute hypoglycemia and hepatorenal damage leading to death (Diwan, 2000). Toxic alkaloids cause liver megalocytosis, proliferation of biliary tract epithelium, liver cirrhosis and nodular hyperplasia (Zeinsteger *et al.*, 2003).

The significant increase in white blood cells count resulting after a daily oral and intraperitoneal administration of aqueous extracts of *B. holstii, P. capense, V. lasiopus, G. parviflora* and *S. asper* at 450, 670 and 1000 mg/kg body weight in normal mice for 28 days indicates a boosted immunity by these extracts (Kaushansky, 1995; Li, Xia and Kuter, 1999). This could be due to tissue damage caused by some constituents of the aqueous plants extracts. This argument is in line with the observed alteration of organ sizes in normal mice orally and intraperitoneally administered with aqueous extracts of *B. holstii, P. capense, V. lasiopus, G. parviflora* and *S. asper* at 450, 670 and 1000 mg/kg body weight for 28 days.

The significant increase in absolute neutrophil, lymphocyte, eosinophil, and monocyte counts on oral and intraperitoneal administration of aqueous extracts of *B. holstii, P. capense, V. lasiopus, G. parviflora* and *S. asper* at 450, 670 and 1000 mg/kg body weight in normal mice daily for 28 days may imply an increased response to bacterial and viral infections, inflammations, and allergens (Howard and Hamilton, 2002; McKnight *et al.,* 1999). A significant increase in absolute neutrophil may indicate activation of neutrophil production leading to an increased response to bacterial infections induced by constituents present in these aqueous plants extracts. A significant increase in absolute lymphocytes (main effectors cells of the immune system) may indicate activation of lymphocyte production leading to an increased response to inflammation induced by toxic constituents in these aqueous plants extracts (McKnight *et al.,* 1999). A significant increase in absolute eosinophil count may indicate activation of eosinophil production leading to an increased response to

allergens and inflammation induced by toxic constituents of these aqueous plants extracts (Howard and Hamilton, 2002). A significant increase in absolute monocyte count may indicate activation of monocyte production leading to an increased response to chronic infection or bone marrow damage induced by toxic constituents of these aqueous plants extracts (Howard and Hamilton, 2002). Increased absolute white blood cell, neutrophil, lymphocyte, eosinophil, and monocyte counts resulting from tissue injury is confirmed by the alterations in organ sizes after a daily oral and intraperitoneal administration of aqueous extracts of *B. holstii*, *P. capense, V. lasiopus, G. parviflora* and *S. asper* at 450, 670 and 1000 mg/kg body weight in normal mice for 28 days.

The increased platelet count (thrombocytosis) in normal mice orally and intraperitoneally administered with aqueous extracts of *B. holstii*, *P. capense, V. lasiopus, G. parviflora* and *S. asper* at 450, 670 and 1000 mg/kg body weight daily for 28 days is associated with bone marrow damage or bleeding disoders induced by toxic consituents in these aqueous extracts.

The antidiabetic and toxicological effects induced by the five aqueous extracts of *B. holstii*, *P. capense, V. lasiopus, G. parviflora* and *S. asper* provide a basis for the rejection of the null hypotheses.

5.2 CONCLUSIONS

In conclusion, the findings of this studuy indicate that:

1. Oral and intraperitoneal administration of the five aqueous extracts of *B. holstii, P. capense, V. lasiopus, G. parviflora* and *S. asper* at 25, 48.4, 93.5, 180.9 and 350 mg/kg body weight in alloxan induced diabetic mice caused significant antidiabetic activity. Further, the oral and the intraperitoneal route were similarly effective in lowering blood glucose levels in alloxan induced diabetic mice. The best antidiabetic aqueous plants extracts were those of *P. capense* (oral), and *V. lasiopus* (intraperitoneal) using the mouse model.
2. Oral and intraperitoneal administration of aqueous extracts of *B. holstii, P. capense, V. lasiopus, G. parviflora* and *S. asper* at 450, 670 and 1000 mg/kg body weight in normal mice daily for 28 days resulted in significant changes in average weekly body weight, percent organ to body weight, hematological and biochemical parameters.
3. The five aqueous extracts of *B. holstii, P. capense, V. lasiopus, G. parviflora* and *S. asper* contained total phenols, flavonoids, tannins, saponins, and alkaloids at various levels. Further, the five aqueous extracts of *B. holstii, P. capense, V. lasiopus, G. parviflora* and *S. asper* contained mineral elements K, Ca, Mn, Fe, Zn, Br, Rb, Cr, Ti, Cu, As, Sr, and Pb at various levels. However, some measured mineral elements such as Ca, K, Mn, Fe, Rb and Br were at levels beyond the recommended daily allowance.
4. The phytochemicals and mineral elements present in the aqueous extracts of *B. holstii, P. capense, V. lasiopus, G. parviflora* and *S. asper* are responsible for the

antidiabetic and toxicological effects observed in this study using the mouse model.

5.3 RECOMMEDATIONS

1. This study recommends continuous use of the aqueous extracts of *Sonchus asper, Vernonia lasiopus, Galinsoga parviflora, Piper capense* and *Berberis holstii* as traditional medicines in the management of diabetes mellitus. However, doses beyond the recommended preliminary testing doses of 50-350 mg/kg body weight be avoided because of their demonstrated toxicological effects.
2. The best plants to use for the traditional management of diabetes based on this study are orally administer aqueous extracts of *P. capense* at **25**-350mg/kg body weight and intraperitoneally administer aqueous extracts of *V. lasiopus* at 25-350mg/kg body weight since they demonstrated the highest blood glucose reduction in alloxan induced diabetic mice.

5.3.1 RECOMMENDATIONS FOR FURTHER RESEARCH

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

2. To establish the mechanism of antidiabetic acitivity and toxicity of the aqueous extracts of *Sonchus asper, Vernonia lasiopus, Galinsoga parviflora, Piper capense* and *Berberis holstii* using the mouse model.

3. To investigate the antidiabetic and toxic effect of the combined dosages of these plants extracts. This will create a rationale for a combination therapy in the

management of diabetes mellitus since it is the mode of administering herbal medicines traditionally by herbal medical practitioners' in their clinical practice.

4. To carry out comprehensive toxicity studies including assessing whether the administration of the aqueous extracts of *Sonchus asper, Vernonia lasiopus, Galinsoga parviflora*, *Piper capense* and *Berberis holstii* at 450, 670 and 1000 mg/kg body weight in mice daily for 28 days alters the histology of tissues/organs and the levels of serum iron, folate and vitamin B_{12}.

5. To isolate and identify the antidiabetic compounds using organic solvents of increasing polarity from the five plants using a bioassay guided approach and assess their mechanism of efficacy and safety or otherwise using molecular approaches such as proteomics and transcriptomics.

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APPENDICES

Appendix 1: Effects of five therapeutic doses of aqueous plant extracts of *Berberis holstii* on percentage change on blood glucose levels in diabetic mice

Treatment	Route	Percentage Glucose change at Varying Times					
		0 hour	2 hour	4 hour	6 hour	8 hour	24 hour
Normal control	Oral	100	100.01 ± 1.92^{befghi}	99.62 ± 2.06^{abefghi}	98.86 ± 2.18^{abefghi}	99.24 ± 1.03^{abefghi}	100.39 ± 1.61^{abfghi}
	IP	100	100.38 ± 0.86^{BDEFGH}	100.38 ± 1.60^{ABDEFGH}	99.62 ± 1.62^{ABDEFGH}	99.62 ± 1.60^{ABDEFGH}	100.38 ± 0.86^{ABDEFGH}
Negative control	Oral	100	106.38 ± 5.23^{bdefgh}	116.83 ± 6.28^{dbefghi}	124.26 ± 6.15^{dbefghi}	133.82 ± 11.02^{dbefghi}	150.49 ± 15.68^{dbefghi}
	IP	100	109.54 ± 2.01^{BDEFGH}	116.61 ± 4.46^{CBDEFGH}	129.40 ± 9.15^{CBDEFGH}	138.61 ± 7.04^{CBDEFGH}	164.53 ± 8.39^{CBDEFGH}
Positive control (glibenclamide)	Oral	100	79.23 ± 5.23^{dag}	54.36 ± 7.36^{da}	42.28 ± 4.40^{da}	37.56 ± 4.97^{da}	60.70 ± 8.48^{da}
Positive control (insulin)	IP	100	51.16 ± 5.43^{CADEGH}	48.14 ± 6.14^{CAD}	41.94 ± 6.07^{CA}	38.78 ± 5.18^{CA}	52.70 ± 8.55^{CADG}
Extract dose (mg/kg body weight)							
25	Oral	100	83.84 ± 9.61^{dagf}	68.38 ± 10.04^{dafg}	50.78 ± 5.55^{daf}	42.08 ± 6.46^{da}	79.37 ± 11.47^{ai}
	IP	100	72.69 ± 3.44^{CABF}	58.58 ± 5.06^{CABEF}	47.42 ± 5.88^{CAF}	40.71 ± 5.74^{CA}	73.62 ± 4.57^{CABH}
48.4	Oral	100	66.77 ± 5.54^{dae}	45.03 ± 7.59^{dae}	$37.73\pm\pm9.29^{dae}$	31.94 ± 8.12^{da}	66.58 ± 11.32^{da}
	IP	100	62.81 ± 5.75^{CAB}	44.70 ± 2.51^{CADGH}	36.53 ± 3.53^{CA}	32.05 ± 3.76^{CA}	60.64 ± 5.27^{CA}
93.5	Oral	100	64.08 ± 8.10^{dabe}	47.48 ± 5.62^{dae}	39.18 ± 4.25^{da}	34.10 ± 2.85^{da}	72.63 ± 6.64^{dai}
	IP	100	57.17 ± 4.00^{CADGH}	41.91 ± 3.65^{CADGH}	34.64 ± 3.54^{CAD}	30.80 ± 2.88^{CA}	64.88 ± 6.13^{CAH}
180.9	Oral	100	73.87 ± 5.51^{da}	56.48 ± 4.43^{da}	40.75 ± 5.51^{da}	39.83 ± 13.67^{da}	70.39 ± 5.86^{dai}
	IP	100	68.11 ± 6.54^{CAB}	54.04 ± 3.94^{CAEF}	40.35 ± 6.04^{CA}	35.10 ± 7.45^{CA}	69.65 ± 8.08^{CABH}
350	Oral	100	76.92 ± 7.74^{da}	58.98 ± 6.87^{da}	46.86 ± 2.71^{da}	34.42 ± 5.22^{da}	46.15 ± 12.63^{daegh}
	IP	100	72.56 ± 6.53^{CABF}	53.75 ± 3.45^{CAEF}	43.99 ± 3.81^{CA}	33.95 ± 5.21^{CA}	47.48 ± 9.52^{CADFG}

Results are expressed as Means ± Standard Deviation for five mice per group. Means accompanied by similar upper case letters and similar lower case letters in the same column are significantly different at $\rho \leq 0.05$ by ANOVA and post ANOVA (Bonferroni-Holm) test. Means for IP administration: $^{C}\rho < 0.05$ with respect to normal control; $^{A}\rho < 0.05$ with respect to diabetic control; $^{B}\rho < 0.05$ with respect to insulin; $^{D}\rho < 0.05$ with respect to 25 mg/kg; $^{E}\rho < 0.05$ with respect to 48.4 mg/kg; $^{F}\rho < 0.05$ with respect to 93.5 mg/kg; $^{G}\rho < 0.05$ with respect to 180 mg/kg; $^{H}\rho < 0.05$ with respect to 350 mg/kg. Means for Oral administration: $^{d}\rho < 0.05$ with respect to normal control; $^{a}\rho < 0.05$ with respect to diabetic control; $^{b}\rho < 0.05$ with respect to glibenclamide $^{e}\rho < 0.05$ with respect to 25 mg/kg; $^{f}\rho < 0.05$ with respect to 48.4 mg/kg; $^{g}\rho < 0.05$ with respect to 93.5 mg/kg; $^{h}\rho < 0.05$ with respect to 180 mg/kg; $^{i}\rho < 0.05$ with respect to 350 mg/kg.

Appendix 2: Effects of five therapeutic doses of aqueous plant extracts of *Piper capense* on percentage change on blood glucose levels in diabetic mice

Treatment	Route	Percentage Glucose change at Varying Times					
		0 hour	2 hour	4 hour	6 hour	8 hour	24 hour
Normal control	Oral	100	100.01±1.94	100.41±2.53^{befghi}	99.63±1.61^{befghi}	100.39±0.87^{abefghi}	100.79±2.22^{ab}
	IP	100	101.14±1.69^{BDEFGH}	100.03±2.72^{ABDEFGH}	99.25±1.71^{ABDEFGH}	100.76±1.05^{ABDEFGH}	101.16±2.19^{ABDEFGH}
Negative control	Oral	100	110.68±6.13^{ei}	121.87±9.49^{befghi}	130.68±12.30^{befghi}	145.40±16.25^{dbefghi}	102.72±16.30^{dbefghi}
	IP	100	107.96±1.68^{BDEFGH}	116.60±1.12^{CBDEFGH}	126.56±5.90^{CBDEFGH}	137.65±4.93^{CBDEFGH}	161.11±9.32^{CBDEFGH}
Positive control (glibenclamide)	Oral	100	70.77±13.59	52.39±9.97^{da}	41.67±7.07^{da}	34.25±7.07^{da}	52.86±8.55^{da}
Positive control (insulin)	IP	100	43.98±3.01^{CADEF}	41.40±2.76^{CAD}	37.81±2.74^{CAH}	35.58±2.16^{CAFGH}	47.80±6.23^{CADE}
Extract dose (mg/kg body weight)							
25	Oral	100	47.70±12.13^{a}	36.25±5.82^{da}	32.47±5.67^{da}	30.02±5.54^{da}	70.78±5.40^{a}
	IP	100	70.26±4.94^{CABEFGH}	53.97±6.20^{CABFGH}	45.08±5.40^{CAFGH}	37.67±4.16^{CAEFGH}	75.25±9.79^{CABH}
48.4	Oral	100	74.01±6.70	55.74±9.16^{da}	43.73±7.98^{da}	36.15±4.93^{da}	95.34±5.59^{a}
	IP	100	55.82±6.08^{CABD}	42.67±5.58^{CA}	35.07±5.63^{CAD}	28.21±6.79^{CADH}	65.99±2.81^{CABH}
93.5	Oral	100	58.66±7.06	40.69±9.05^{da}	32.92±5.92^{da}	27.48±5.49^{da}	62.41±10.08^{a}
	IP	100	55.59±1.37^{CABD}	38.09±7.49^{CAD}	30.72±6.89^{CAD}	23.37±3.19C^{ABD}	60.09±6.63^{CAH}
180.9	Oral	100	59.09±18.43	45.01±14.36^{da}	38.69±11.56^{da}	33.40±9.20^{da}	66.83±13.36^{a}
	IP	100	51.76±8.48^{CAD}	36.78±7.27^{CAD}	29.76±6.19^{CAD}	24.70±3.82^{CABD}	60.10±5.36^{CAH}
350	Oral	100	48.21±22.17^{a}	40.07±2.40^{da}	25.58±3.23^{da}	21.47±2.96^{da}	57.57±6.45^{a}
	IP	100	48.66±7.12^{CAD}	32.22±5.28^{CAD}	25.39±5.47^{CABD}	18.97±4.24^{CABDE}	40.98±10.05^{CADEFG}

Results are expressed as Means ± Standard Deviation for five mice per group. Means accompanied by similar upper case letters and similar lower case letters in the same column are significantly different at $\rho \leq 0.05$ by ANOVA and post ANOVA (Bonferroni-Holm) test. Means for IP administration: $^{C}\rho < 0.05$ with respect to normal control; $^{A}\rho < 0.05$ with respect to diabetic control; $^{B}\rho < 0.05$ with respect to insulin; $^{D}\rho < 0.05$ with respect to 25 mg/kg; $^{E}\rho < 0.05$ with respect to 48.4 mg/kg; $^{F}\rho < 0.05$ with respect to 93.5 mg/kg; $^{G}\rho < 0.05$ with respect to 180 mg/kg; $^{H}\rho < 0.05$ with respect to 350 mg/kg. Means for Oral administration: $^{d}\rho < 0.05$ with respect to normal control; $^{a}\rho < 0.05$ with respect to diabetic control; $^{b}\rho < 0.05$ with respect to glibenclamide $^{e}\rho < 0.05$ with respect to 25 mg/kg; $^{f}\rho < 0.05$ with respect to 48.4 mg/kg; $^{g}\rho < 0.05$ with respect to 93.5 mg/kg; $^{h}\rho < 0.05$ with respect to 180 mg/kg; $^{i}\rho < 0.05$ with respect to 350 mg/kg.

Appendix 3: Effects of five therapeutic doses of aqueous plant extracts of *Vernonia lasiopus* on percentage change on blood glucose levels in diabetic mice

Treatment	Route	Percentage Glucose change at Varying Times					
		0 hour	2 hour	4 hour	6 hour	8 hour	24 hour
Normal control	Oral	100	101.19±1.70^{befghi}	101.58±2.16^{abefghi}	100.80±2.25^{abefghi}	99.64±1.61^{abefghi}	102.37±3.29^{abefghi}
	IP	100	100.38±0.86^{BDEFGH}	99.62±1.62^{ABDEFGH}	100.00±1.36^{ABDEFGH}	99.62±1.62^{ABDEFGH}	101.16±1.14^{ABDEFGH}
Negative control	Oral	100	113.14±4.42^{befghi}	121.03±5.97^{dbefghi}	129.45±6.06^{dbefghi0}	145.72±7.46^{dbefghi}	167.64±12.08^{dbefghi}
	IP	100	108.98±3.52^{BDEFGH}	119.34±9.02^{CBDEFGH}	130.03±14.07C^{BDEFGH}	139.32±17.01^{CBDEFGH}	166.19±14.12^{CBDEFGH}
Positive control (glibenclamide)	Oral	100	67.01±9.75^{da}	49.79±6.97^{da}	38.83±4.65^{da}	32.40±4.91^{da}	50.19±2.96^{dae}
Positive control (insulin)	IP	100	41.55±5.19^{CA}	37.99±6.67^{CA}	36.41±6.13^{CA}	33.76±5.98^{CA}	44.72±7.23^{CAD}
Extract dose (mg/kg body weight)							
25	Oral	100	75.26±7.40^{da}	57.21±6.46^{da}	49.20±4.83^{dafgh}	43.38±6.83^{dafghi}	70.68±6.67^{dabghi}
	IP	100	51.83±9.01^{CA}	41.17±4.15^{CA}	32.33±1.84^{CA}	29.89±1.44^{CA}	70.97±5.04^{CABH}
48.4	Oral	100	63.81±10.00^{da}	45.27±5.05^{da}	33.21±6.34^{dae}	28.55±6.27^{dae}	57.78±10.67^{dai}
	IP	100	40.00±9.90^{CA}	32.57±9.89^{CA}	24.18±7.10^{CA}	21.54±6.10^{CA}	57.04±6.90^{CA}
93.5	Oral	100	67.09±4.45^{da}	46.52±9.73^{da}	31.40±7.76^{dae}	25.56±4.53^{dae}	54.76±3.28^{daei}
	IP	100	44.11±4.16^{CA}	37.27±3.76^{CA}	30.45±4.40^{CA}	26.39±2.82^{CA}	51.88±5.65^{CA}
180.9	Oral	100	60.03±1.94^{da}	48.30±3.26^{da}	38.79±5.00^{da}	30.89±5.56^{dae}	49.44±7.70^{dae}
	IP	100	50.07±15.17^{CA}	35.78±8.28^{CA}	26.10±3.44^{CA}	21.68±3.58^{CA}	53.47±8.60^{CA}
350	Oral	100	69.72±13.32^{da}	51.74±5.68^{da}	38.47±7.68^{da}	29.80±2.91^{dae}	35.35±5.77^{daefg}
	IP	100	53.39±16.33^{CA}	36.31±7.33^{CA}	27.23±4.62^{CA}	23.37±4.79^{CA}	44.03±7.79^{CAD}

Results are expressed as Means ± Standard Deviation for five mice per group. Means accompanied by similar upper case letters and similar lower case letters in the same column are significantly different at $\rho \leq 0.05$ by ANOVA and post ANOVA (Bonferroni-Holm) test. Means for IP administration: $^{C}\rho < 0.05$ with respect to normal control; $^{A}\rho < 0.05$ with respect to diabetic control; $^{B}\rho < 0.05$ with respect to insulin; $^{D}\rho < 0.05$ with respect to 25 mg/kg; $^{E}\rho < 0.05$ with respect to 48.4 mg/kg; $^{F}\rho < 0.05$ with respect to 93.5 mg/kg; $^{G}\rho < 0.05$ with respect to 180 mg/kg; $^{H}\rho < 0.05$ with respect to 350 mg/kg. Means for Oral administration: $^{d}\rho < 0.05$ with respect to normal control; $^{a}\rho < 0.05$ with respect to diabetic control; $^{b}\rho < 0.05$ with respect to glibenclamide $^{e}\rho < 0.05$ with respect to 25 mg/kg; $^{f}\rho < 0.05$ with respect to 48.4 mg/kg; $^{g}\rho < 0.05$ with respect to 93.5 mg/kg; $^{h}\rho < 0.05$ with respect to 180 mg/kg; $^{i}\rho < 0.05$ with respect to 350 mg/kg.

Appendix 4: Effects of five therapeutic doses of aqueous plant extracts of *Galinsoga parviflora* on percentage change on blood glucose levels in diabetic mice

Treatment	Route	Percentage Glucose change at Varying Times					
		0 hour	2 hour	4 hour	6 hour	8 hour	24 hour
Normal control	Oral	100	100.00±1.90^{befghi}	99.62±1.60^{abefghi}	98.86±1.69^{abefghi}	100.38±1.59^{abefghi}	99.62±2.07^{abefghi}
	IP	100	100.74±1.01^{BDEFGH}	100.37±1.57^{ABDEFGH}	99.62±0.84^{ABDEFGH}	98.51±0.83^{ABDEFGH}	99.24±1.03^{ABDEFGH}
Negative control	Oral	100	109.63±6.40^{befghi}	119.90±8.01^{dbefghi}	131.94±7.57^{dbefghi}	144.46±10.63^{dbefghi}	152.80±15.24^{dbefghi}
	IP	100	108.84±3.02^{BDEFGH}	115.09±2.71^{CBDEFGH}	124.13±8.08^{CBDEFGH}	134.32±7.65^{CBDEFGH}	155.28±20.18^{CBDEFGH}
Positive control (glibenclamide)	Oral	100	70.15±10.11^{da}	54.48±7.63^{da}	42.11±6.83^{da}	34.87±5.27^{da}	53.97±4.83^{dai}
Positive control (insulin)	IP	100	43.34±2.22^{CA}	39.16±3.89^{CA}	34.62±4.52^{CA}	32.16±4.04^{CA}	49.68±6.25^{CA}
Extract dose (mg/kg body weight)							
25	Oral	100	75.65±9.75^{da}	56.88±8.61^{da}	47.70±10.74^{dafh}	41.62±9.71^{dafgh}	71.53±11.62^{dahi}
	IP	100	54.64±12.55^{CA}	32.02±5.30^{CA}	29.12±5.12^{CA}	26.03±4.31^{CA}	61.48±5.63^{CAH}
48.4	Oral	100	65.78±7.07^{da}	44.50±1.75^{dai}	32.89±3.13^{dae}	27.25±4.98^{dae}	58.58±8.57^{dai}
	IP	100	43.35±4.39^{CA}	31.53±3.38^{CAG}	27.54±2.45^{CA}	24.76±1.85^{CA}	60.24±5.33^{CAH}
93.5	Oral	100	69.06±2.27^{da}	50.92±7.26^{da}	34.06±4.45^{da}	25.62±4.80^{dae}	56.13±5.32^{dai}
	IP	100	47.02±9.60^{CA}	36.02±8.49^{CA}	32.37±6.42^{CA}	27.57±5.12^{CA}	57.06±4.56^{CA}
180.9	Oral	100	62.30±10.36^{da}	43.62±5.70^{dai}	31.92±4.07^{dae}	25.92±3.72^{dae}	49.18±3.43^{dae}
	IP	100	51.50±9.77^{CA}	42.01±5.59^{CAE}	35.32±4.92^{CA}	30.97±5.91^{CA}	50.68±7.11^{CA}
350	Oral	100	72.74±4.31^{da}	59.36±8.93^{dafh}	40.78±9.47^{da}	32.22±5.54^{da}	33.06±3.43^{dabefg}
	IP	100	46.59±9.10^{CA}	34.97±3.40^{CA}	29.01±3.42^{CA}	23.55±1.67^{CA}	39.89±2.67^{CADE}

Results are expressed as Means ± Standard Deviation for five mice per group. Means accompanied by similar upper case letters and similar lower case letters in the same column are significantly different at $\rho \leq 0.05$ by ANOVA and post ANOVA (Bonferroni-Holm) test. Means for IP administration: $^{C}\rho < 0.05$ with respect to normal control; $^{A}\rho < 0.05$ with respect to diabetic control; $^{B}\rho < 0.05$ with respect to insulin; $^{D}\rho < 0.05$ with respect to 25 mg/kg; $^{E}\rho < 0.05$ with respect to 48.4 mg/kg; $^{F}\rho < 0.05$ with respect to 93.5 mg/kg; $^{G}\rho < 0.05$ with respect to 180 mg/kg; $^{H}\rho < 0.05$ with respect to 350 mg/kg. Means for Oral administration: $^{d}\rho < 0.05$ with respect to normal control; $^{a}\rho < 0.05$ with respect to diabetic control; $^{b}\rho < 0.05$ with respect to glibenclamide $^{e}\rho < 0.05$ with respect to 25 mg/kg; $^{f}\rho < 0.05$ with respect to 48.4 mg/kg; $^{g}\rho < 0.05$ with respect to 93.5 mg/kg; $^{h}\rho < 0.05$ with respect to 180 mg/kg; $^{i}\rho < 0.05$ with respect to 350 mg/kg.

Appendix 5: Effects of five therapeutic doses of aqueous plant extracts of *Sonchus asper* on percentage change on blood glucose levels in diabetic mice

Treatment	Route	Percentage Glucose change at Varying Times					
		0 hour	2 hour	4 hour	6 hour	8 hour	24 hour
Normal control	Oral	100	100.78 ± 1.73^{befghi}	100.42 ± 2.17^{abefghi}	101.20 ± 3.01^{abefghi}	100.80 ± 2.24^{abefghi}	101.16 ± 1.06^{abefghi}
	IP	100	100.38 ± 1.60^{BDEFGH}	100.01 ± 1.89^{ABDEFGH}	100.01 ± 1.34^{ABDEFGH}	100.78 ± 1.06^{ABDEFGH}	101.17 ± 2.18^{ABDEFGH}
Negative control	Oral	100	110.05 ± 3.66^{befghi}	117.40 ± 3.86^{dbefghi}	127.05 ± 5.43^{dbefghi}	139.06 ± 7.54^{dbefghi}	153.33 ± 5.88^{dbefghi}
	IP	100	108.55 ± 1.46^{BDEFGH}	114.10 ± 2.94^{CBDEFGH}	120.54 ± 1.99^{CBDEFGH}	128.23 ± 3.57^{CBDEFGH}	144.09 ± 8.78^{CBDEFGH}
Positive control (glibenclamide)	Oral	100	77.06 ± 8.06^{da}	58.95 ± 6.37^{da}	44.08 ± 4.25^{da}	36.52 ± 6.52^{da}	57.46 ± 6.55^{da}
Positive control (insulin)	IP	100	45.49 ± 2.61^{CADEFGH}	42.03 ± 2.48^{CAEFGH}	37.47 ± 3.62^{CA}	34.94 ± 3.05^{CA}	49.13 ± 4.31^{CAEFG}
Extract dose (mg/kg body weight)							
25	Oral	100	75.84 ± 10.47^{da}	57.46 ± 5.10^{da}	46.10 ± 8.11^{da}	40.24 ± 7.79^{da}	62.40 ± 14.49^{da}
	IP	100	72.30 ± 2.60^{CAB}	52.32 ± 5.04^{CA}	44.92 ± 4.55^{CA}	38.86 ± 4.86^{CA}	60.94 ± 5.24^{CA}
48.4	Oral	100	66.88 ± 4.45^{da}	60.99 ± 4.41^{da}	48.77 ± 3.78^{da}	38.72 ± 5.38^{da}	68.13 ± 4.76^{da}
	IP	100	64.38 ± 6.16^{CAB}	53.16 ± 4.83^{CAB}	44.66 ± 6.43^{CA}	40.15 ± 5.54^{CA}	65.11 ± 3.64^{CABH}
93.5	Oral	100	78.60 ± 9.17^{da}	61.19 ± 4.87^{da}	45.84 ± 5.12^{da}	37.78 ± 3.60^{da}	69.60 ± 3.91^{da}
	IP	100	69.27 ± 2.41^{CAB}	54.35 ± 3.66^{CAB}	44.79 ± 5.63^{CA}	39.04 ± 3.02^{CA}	65.45 ± 3.57^{CABH}
180.9	Oral	100	76.51 ± 6.74^{da}	59.20 ± 6.56^{da}	44.73 ± 6.12^{da}	38.28 ± 6.60^{da}	67.41 ± 9.33^{da}
	IP	100	71.98 ± 6.43^{CAB}	53.35 ± 3.12^{CAB}	42.97 ± 4.83^{CA}	36.56 ± 2.15^{CA}	64.52 ± 4.31^{CABH}
350	Oral	100	78.11 ± 6.38^{da}	57.09 ± 8.21^{da}	45.98 ± 4.93^{da}	38.63 ± 6.66^{da}	52.09 ± 12.31^{da}
	IP	100	69.56 ± 10.65^{CAB}	52.84 ± 10.25^{CAB}	42.61 ± 4.71^{CA}	36.60 ± 2.15^{CA}	49.35 ± 8.56^{CAEFGH}

Results are expressed as Means ± Standard Deviation for five mice per group. Means accompanied by similar upper case letters and similar lower case letters in the same column are significantly different at $\rho \leq 0.05$ by ANOVA and post ANOVA (Bonferroni-Holm) test. Means for IP administration: $^{C}\rho < 0.05$ with respect to normal control; $^{A}\rho < 0.05$ with respect to diabetic control; $^{B}\rho < 0.05$ with respect to insulin; $^{D}\rho < 0.05$ with respect to 25 mg/kg; $^{E}\rho < 0.05$ with respect to 48.4 mg/kg; $^{F}\rho < 0.05$ with respect to 93.5 mg/kg; $^{G}\rho < 0.05$ with respect to 180 mg/kg; $^{H}\rho < 0.05$ with respect to 350 mg/kg. Means for Oral administration: $^{d}\rho < 0.05$ with respect to normal control; $^{a}\rho < 0.05$ with respect to diabetic control; $^{b}\rho < 0.05$ with respect to glibenclamide $^{e}\rho < 0.05$ with respect to 25 mg/kg; $^{f}\rho < 0.05$ with respect to 48.4 mg/kg; $^{g}\rho < 0.05$ with respect to 93.5 mg/kg; $^{h}\rho < 0.05$ with respect to 180 mg/kg; $^{i}\rho < 0.05$ with respect to 350 mg/kg.

Appendix 6: Research authorization

KENYATTA UNIVERSITY
GRADUATE SCHOOL

E-mail: kubps@yahoo.com
dean-graduate@ku.ac.ke
Website: www.ku.ac.ke

P.O. Box 43844, 00100
NAIROBI, KENYA
Tel. 8710901 Ext. 57530

Our Ref: 156/CE/26258/11

Date: 18th November, 2015

The Principal Secretary,
Higher Education, Science & Technology,
P.O. Box 30040,
NAIROBI

Dear Sir/Madam,

RE: RESEARCH AUTHORIZATION FOR MS.KIMANI L. NJERI-REG.NO.156/CE/26258/11

I write to introduce Ms. Njeri who is a Postgraduate Student of this University. He is registered for a M.Sc. degree programme in the **Department of Biochemistry & Biotechnology in the School of Pure & Applied Sciences.**

Ms. Njeri intends to conduct research for a thesis Proposal entitled **"Antidiabetic Activity of Aqueous Extracts of *Piper Capense, Berberis Holstii, Sonchus Asper, Vernonia Lasiopus* and *Galinsoga Parviflora* in Alloxan-Induced Diabetic Albino Mice"**

Any assistance given will be highly appreciated.

Yours faithfully,

MRS. LUCY N. MBAABU
FOR: DEAN, GRADUATE SCHOOL

RM/cao

Appendix 7: Registration of the MSc Research Proposal

KENYATTA UNIVERSITY
GRADUATE SCHOOL

E-mail: kubps@yahoo.com
dean-graduate@ku.ac.ke
Website: www.ku.ac.ke

P.O. Box 43844, 00100
NAIROBI, KENYA
Tel. 810901 Ext. 57530

Internal Memo

FROM: Dean, Graduate School

DATE: 18th November, 2015

TO: **Ms. Kimani Lucy Njeri**
C/o Biochemistry & Biotechnology Dept.
KENYATTA UNIVERSITY

REF: 156/CE/26258/11

SUBJECT: APPROVAL OF RESEARCH PROPOSAL

We acknowledge the receipt of your revised Research Proposal entitled **"Antidiabetic Activity of Aqueous Extracts of *Piper Capense, Berberis Holstii, Sonchus Asper, Vernonia Lasiopus* and *Galinsoga Parviflora* in Alloxan-Induced Diabetic Albino Mice"** as per recommendations raised by the Graduate School Board of 11th March, 2015.

You may now proceed with your Data collection, subject to clearance with the Director General, National Commission for Science, Technology & Innovation

As you embark on your data collection, please note that you will be required to submit to Graduate School completed supervision Tracking Forms per semester. The form has been developed to replace the progress Report Forms. The Supervision Tracking Forms are available at the University's Website under Graduate School webpage downloads.

REUBEN MURIUKI
FOR: DEAN, GRADUATE SCHOOL

c.c. Chairman, Biochemistry & Biotechnology Dept.

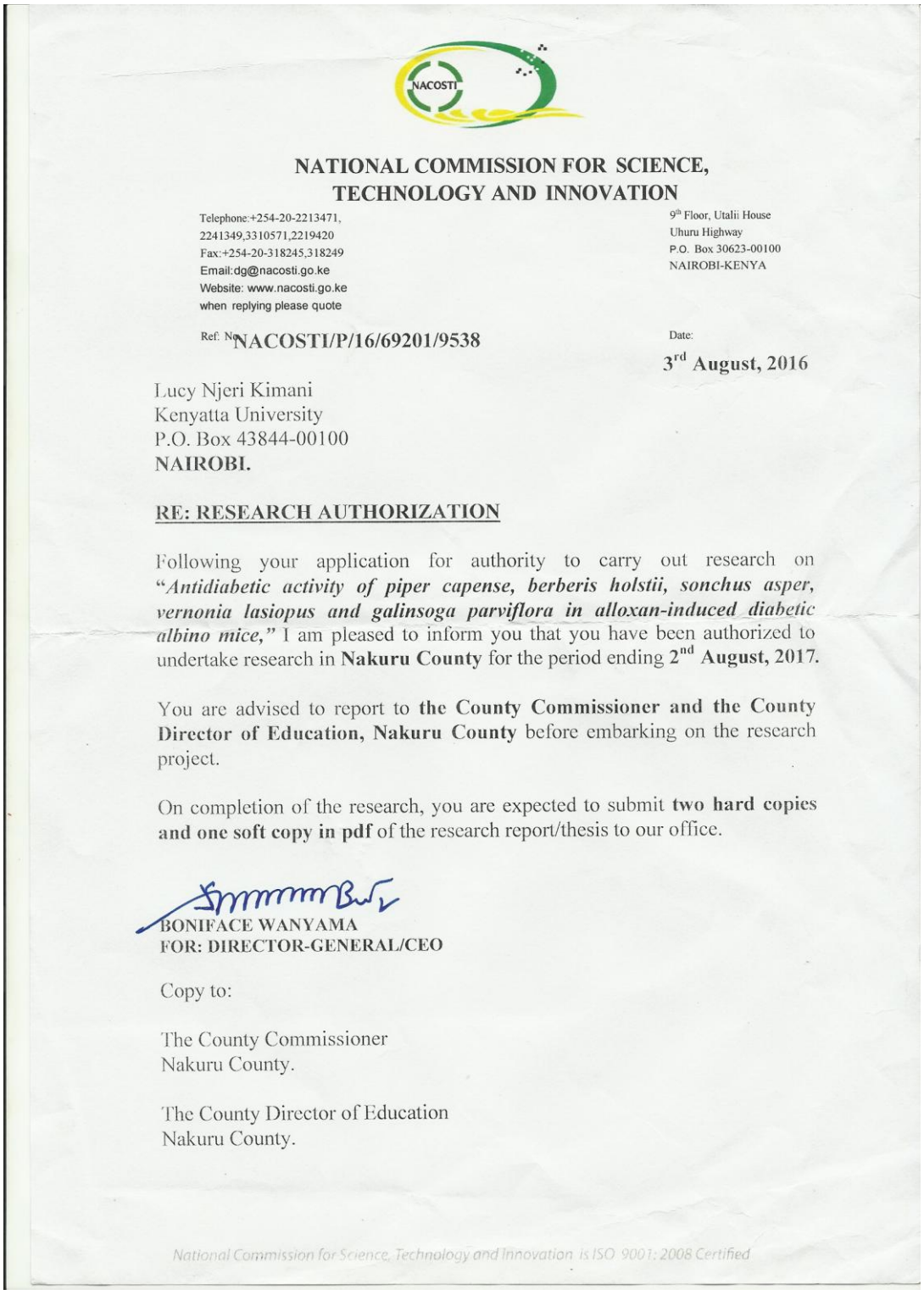
Supervisors:

1. Prof Eliud Njagi
   C/o Biochemistry & Biotechnology Sciences
   KENYATTA UNIVERSITY

2. Dr. George O. Orinda
   C/o Biochemistry & Biotechnology Dept.
   KENYATTA UNIVERSITY

RM/cao

Apendix 8: Research Authorization by NACOSTI



NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY AND INNOVATION

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NAIROBI-KENYA

Ref. No. **NACOSTI/P/16/69201/9538**

Date: **3rd August, 2016**

Lucy Njeri Kimani
Kenyatta University
P.O. Box 43844-00100
NAIROBI.

RE: RESEARCH AUTHORIZATION

Following your application for authority to carry out research on ***"Antidiabetic activity of piper capense, berberis holstii, sonchus asper, vernonia lasiopus and galinsoga parviflora in alloxan-induced diabetic albino mice,"*** I am pleased to inform you that you have been authorized to undertake research in **Nakuru County** for the period ending **2nd August, 2017.**

You are advised to report to **the County Commissioner and the County Director of Education, Nakuru County** before embarking on the research project.

On completion of the research, you are expected to submit **two hard copies and one soft copy in pdf** of the research report/thesis to our office.

BONIFACE WANYAMA
FOR: DIRECTOR-GENERAL/CEO

Copy to:

The County Commissioner
Nakuru County.

The County Director of Education
Nakuru County.

Apendix 9: Identification of Plants by National Museums of Kenya

NATIONAL MUSEUMS OF KENYA

WHERE HERITAGE LIVES ON

The East African Herbarium
P.O. Box 45166 00100 Nairobi, Kenya
Telephone: 3743513, 3742131/4 ext 2274
Fax: 3741424
E-Mail: botany@museums.or.ke

REF: NMK/BOT/CTX/1/2

3rd September, 2013

Lucy Kimani
P.O. Box
Nairobi
Tel: 0723688287

Dear M/s Kimani

PLANT IDENTITY

The plants specimens that you brought to us for identification have been determined as follows:

1. *Galinsoga parviflora* Cav.Plate, Family: Asteraceae
2. *Piper capense* L. Family: Piperaceae
3. *Sonchus asper* (L.) Hill Family: Asteraceae
4. *Vernonia lasiopus* Family: Asteraceae
5. *Berberis holstii* Engl. Family: Berberdaceae

Yours sincerely,

Mathias Mbale
For: Head, Botany Department